

Poster Session II

December 7, 2010 5:30PM–7:30 PM

1. D-cycloserine Improves the Impaired Sociability of the Balb/c Mouse

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Background: The genetically-inbred Balb/c mouse strain shows evidence of impaired sociability in a standard paradigm and is hypersensitive to behavioral effects of MK-801 (dizocilpine), a noncompetitive NMDA receptor antagonist. Specifically, relative to 8 week-old male outbred Swiss-Webster mice, 8 week-old male Balb/c mice show diminished locomotor activity and spend less time in the vicinity of an enclosed 4 week-old male ICR stimulus mouse in a 5-minute period and, when allowed to interact freely with the stimulus mouse for 5 minutes, make fewer discrete episodes of social approach and show suppression of its locomotor activity. Additionally, MK-801 elicits more irregular episodes of intense jumping behavior, referred to as “popping,” and more intense mouse circling behavior, and antagonizes electrically-precipitated seizures more potently, on a mg/kg basis, in the Balb/c strain, relative to other genetically-inbred and outbred mouse strains. Importantly, a mutant mouse strain with diminished expression of the NR1 subunit of the NMDA receptor (approximately 5–10% of normal levels) is impaired on standardized measures of mouse sociability; thus, diminished endogenous tone of NMDA receptor-mediated neurotransmission is associated with impaired sociability of the mouse. Because the Balb/c mouse strain is impaired in its sociability and has an altered endogenous tone of NMDA receptor-mediated neurotransmission, we explored the effect of D-cycloserine (320 mg/kg, ip), a partial glycine agonist that binds to the obligatory co-agonist glycine binding site on the NMDA receptor, on the sociability of this strain in a standard paradigm.

Methods: D-Cycloserine (320 mg/kg or saline vehicle) was injected ip 20 minutes prior to testing sociability. The locomotor activity and discrete episodes of social approach in a 5-minute period of 8-week-old “test” mice (i.e., Balb/c or Swiss-Webster) in the presence of an enclosed 4-week-old male stimulus mouse (ICR) were measured. Thereafter, measures of social approach and locomotion while the “test” and “stimulus” mice were allowed to interact freely were recorded.

Results: Locomotor activity/5 min of the Balb/c strain was significantly reduced when the stimulus mouse was enclosed and when “test” and “stimulus” mice were allowed to interact freely, relative to the Swiss-Webster strain. Relative to the vehicle only condition, treatment of Balb/c mice with D-cycloserine (320 mg/kg, ip) 20 minutes before testing in the sociability apparatus significantly increased their locomotor activity in the presence of an enclosed ICR social stimulus mouse ($p < 0.001$) and when allowed to interact freely with ICR social stimulus mice ($p < 0.001$); locomotor activity of Balb/c mice treated with D-cycloserine did not differ from saline-treated Swiss-Webster mice in these two conditions. Similarly, D-cycloserine (320 mg/kg, ip) restored the number of discrete episodes of social approach/5 min of the Balb/c strain when allowed to interact freely with social stimulus mice ($p < 0.001$), raising the number of social approaches to those shown by saline-treated Swiss-Webster mice.

Discussion: The current results complement the clinical trial data of Posey and colleagues (2004), supporting continued exploration of targeted NMDA receptor agonist interventions for psychiatric disorders manifesting impaired sociability, such as schizophrenia and autism spectrum disorders. Further, the current data replicate and extend earlier work on the impaired sociability of the Balb/c mouse strain. In fact, because this genetically-inbred mouse strain is hypersensitive to behavioral effects of MK-801, a noncompetitive NMDA receptor antagonist, it may be an ideal strain in which to study

the effects of targeted NMDA receptor interventions on sociability in a standardized paradigm.

Disclosure: S. Deutsch: None. J. Burket: None. L. Jacome: None. W. Cannon: None. A. Herndon: None.

2. Serotonin Systems and Modulation in Dopamine Transporter (DAT)-Deficient Mice

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Background: Serotonergic compounds, serotonin transporter depletion and serotonin receptor alterations alter baseline and/or drug-induced phenotypes expressed by dopamine transporter (DAT)-deficient mice, adding to abundant evidence for interactions between serotonergic and dopaminergic systems.

Methods: We now report studies that confirm and extend evidence for selective influences of pharmacological alterations in the serotonin system in DAT wildtype (+/+), heterozygous (+/-) and knockout (-/-) mice.

Results: As previously reported, DAT -/- mice were hyperactive in the open field, traveling ~3x as far as DAT +/+ mice; intermediate phenotypes were displayed by DAT +/- mice. DAT -/- mice traveled at higher velocities and displayed different locomotor patterns when assessed for features that included meandering and turn angles. In the marble burying test, DAT -/- mice buried significantly fewer marbles than DAT +/+ or +/- mice. The 5-HT1A agonist 8-OH-DPAT induced its distinctive serotonin syndrome in wildtype mice, but overall serotonin syndrome scores were greater in DAT -/- mice. Several individual behaviors that contribute to this syndrome, including head weaving and forepaw treading, were also significantly increased, perhaps suggesting increased postsynaptic 5-HT1A receptor function. Although previous work showed that the 5-HT2A antagonist M100907 reversed several behavioral deficits in DAT -/- mice, DOI-induced head twitches were unchanged in DAT -/- mice, suggesting at least some intact 5-HT2A receptor function. HPLC analyses of monoamines and metabolites in brain regions revealed decreased dopamine, increased dopamine/DOPAC ratios, small differences in tissue levels of serotonin and no genotypic differences in levels of 5-HIAA or norepinephrine in DAT -/- mice.

Discussion: These findings extend the behavioral and biochemical phenotypes of DAT-deficient mice, confirm hyperactivity and stereotypy in these mice, support alterations in the phenotypes of DAT -/- mice by serotonergic compounds, but indicate relatively intact serotonin levels and functions of several serotonin receptors - the one notable exception may be postsynaptic 5-HT1A receptors - in these interesting mice. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

Disclosure: M. Fox: None. M. Panessiti: None. F. Hall: None. G. Uhl: None. D. Murphy: None.

3. The Puzzle Box as Simple and Efficient Behavioral Test for Impairments of General Cognition and Executive Functions in Mouse Models of Schizophrenia

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Background: In the light of the current breakthroughs that are unraveling molecular and genetic underpinnings of schizophrenia, it becomes indispensable to establish and/or refine new or currently available analytical tools in animal models. Cognitive symptoms of schizophrenia that primarily depend on the prefrontal cortex, and to

some extent also on the hippocampus, i.e. attention deficits, working memory and executive function impairments, are important features of chronic manifestation of schizophrenia. Various behavioral rodent models have therefore been used when attempting to find animal correlates of cognitive symptoms, most of which require extensive labor and time, to name only few of their limitations. We tried here to establish a simple but informative test on executive functions in mice in different schizophrenia models.

Methods: The puzzle box is a behavioral test developed in rodents, in which subjects are presented with different problems of increasing difficulties, and are expected to solve the problems during a limited lapse of time. The arena consists of a white Plexiglas box divided by a removable barrier into two separate compartments: a big and brightly-lit start zone (58 cm long, 28 cm wide), and a smaller protected goal zone (15 cm long, 28 cm wide) containing sawdust and cardboard. Mice are trained to escape the start zone through an underpass (~4 cm wide) located under a wall separating both zones. They have to undergo a total of nine trials (T1-T9) over 3 consecutive days (3 trials per day) with increasing difficulty of passing the underpass. The underpass is first marked and open (T1), then only open (T2-T4), then filled with sawdust (T5-T7), then closed by a plug (T8-T9). Thus, at days 2 and 3, the difficulty of the first trial corresponds to that of the last trial of the day before, while the second trial each day (T2, T5, T8) always increases the difficulty compared to the preceding one.

Results: We used five different mouse models of schizophrenia, namely mice with prefrontal cortex and hippocampus lesions, respectively, mice treated sub-chronically with the NMDA-antagonist MK-801, mice constitutively lacking the GluR-1 subunit of AMPA-receptors (GluR-1 knockout mice), and mice over-expressing the D2 dopamine receptor in the striatum. Compared to their corresponding experimental controls, all mice models used here demonstrated altered executive functions though to an extent that (not surprisingly) varied between the different models. Strongest deficits were observed in hippocampal lesioned mice and GluR-1 knockout mice, while subtle but specific deficits (particularly in advanced trial 8) were found in MK-801 treated mice and in animals over-expressing D2 receptors.

Discussion: With this report we demonstrate face validity of the puzzle box as a behavioral screening tool for executive functions in general and for schizophrenia mouse models in particular. Interestingly, among the various models studied here, specific behavioral deficits were also found in two models with previously demonstrated deficits of prefrontal cortical functions, i.e. one lesion model and one transgenic model (D2 receptor overexpressing mice). Additional experiments will be performed in an effort to rescue the observed deficiencies in different models by classical and atypical antipsychotic drugs.

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4. Chronic Phenytoin Reverses Stress Enhanced Renewal but not Reinstatement of Conditioned Fear in an Animal Model of Post-Traumatic Stress Disorder

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Background: Despite a number of successful interventions, return of fear (ROF) in patients who suffer from anxiety disorders such as post-traumatic stress disorder (PTSD) remains a significant problem. ROF can be precipitated by re-exposure to a traumatic context (fear renewal) or by reminders salient to the traumatic event itself (fear reinstatement) (Hermans et al. 2005; Effting and Kindt 2007). Preliminary human research suggests that PTSD patients may have alterations in fear conditioning processes (Garfinkel et al, 2009) that contribute to their persistent re-experiencing of trauma-based fear, perhaps via effects on renewal and reinstatement. Animal models may be able to shed light on the underlying neurobiological basis of these

changes. Phenytoin (PHE) is an anticonvulsant that stabilizes excitable membrane activity and has some efficacy in treating PTSD (Bremner et al. 2005), possibly via effects on glutamatergic transmission. We utilized a validated animal model of PTSD - single prolonged stress (SPS, Liberzon et al. 1997; 1999) - to study the impact of stress/trauma on fear renewal and reinstatement and the effects of chronic PHE on these phenomena.

Methods: Thirty-two male Sprague Dawley rats were subjected to SPS or a control procedure. SPS rats were restrained for two hours, followed by 20 minutes of forced swimming. After 15 minutes of recuperation they were exposed to ether until anaesthetized. Rats were then administered PHE (subcutaneous injection, 40 mg/kg) or saline, once a day for seven days. All rats underwent fear conditioning, fear extinction, and renewal testing followed by a reminder shock and a test of reinstatement. On day one, rats were fear conditioned, receiving five tone presentations (10 s; 80 dB; 2 kHz) that co-terminated with a footshock (1 s; 1 mA). On day two, rats received 30 extinction training trials in a different context. On day three, rats were returned to the conditioning context and tested for fear renewal. Three minutes after the last tone presentation, they were exposed to a single footshock. On day four, the rats were tested for reinstatement of fear in the extinction context. Expression of fear was measured using percentage of total time observed spent freezing.

Results: Neither drug treatment with PHE nor SPS affected the acquisition of conditioned fear ($F(1, 27) = 0.14$, $p = 0.71$ and $F(1, 27) = 1.16$, $p = 0.29$). Extinction learning was also unaffected by drug treatment and stress condition ($F(1, 27) = 0.41$, $p = 0.53$ and $F(1, 27) = 1.09$, $p = 0.31$). SPS facilitated renewal of fear following re-exposure to the conditioning context ($F(1, 27) = 5.75$, $p = 0.023$), an effect which was reversed by treatment with PHE ($F(1, 27) = 7.22$, $p = 0.012$). SPS also enhanced the reinstatement of conditioned fear following a single reminder shock ($F(1, 27) = 7.48$, $p = 0.011$) but drug treatment did not alter this effect ($F(1, 27) = 0.65$, $p = 0.43$).

Discussion: These data suggest that SPS affects fear conditioning processes such that fear memories are rendered more likely to be reactivated following extinction. We demonstrate that both context-induced renewal of fear, and sensitivity to trauma reminders are facilitated by the earlier trauma/stress exposure, despite unaltered fear conditioning and extinction. Furthermore, these findings suggest that SPS-enhanced renewal, but not reinstatement, may be dependent upon glutamatergic activity in the days following SPS, because treatment with PHE blocked SPS-induced increases in fear renewal. These results suggest that ROF via renewal and reinstatement are both enhanced by prior stress, but are pharmacologically dissociable. Treatment with compounds that alter excitatory neural transmission may disrupt some but not all ROF processes.

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5. Mk801 Disrupts Associative Learning in Zebrafish

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Background: The mechanisms of learning and memory have been thoroughly investigated using numerous laboratory model organisms and hundreds of important molecular players involved in these processes have already been identified. However, according to some estimates, 10-15 thousand genes may underlie neural plasticity in general and learning and memory in particular. To tackle this complexity, comprehensive and systematic analyses of all these molecular players are needed. Such comprehension may be achieved, for example, by extensive drugs screens or large scale mutation (forward genetic) screens. However, these screens are expensive and slow with traditional laboratory organisms including the mouse and the rat. The zebrafish, a simple vertebrate, may offer a feasible alternative. It is particularly amenable for high throughput screening because of its small size and prolific nature. Furthermore, a powerful

genetic tool set has been developed for zebrafish and thus identification of novel genes using forward genetic approaches as well as functional characterization of known genes using reverse genetic methods are possible. Psychopharmacological approaches are also on the rise for zebrafish. Due to the high amino-acid sequence homologies especially between the functionally relevant domains of zebrafish and mammalian proteins, investigators have been able to successfully utilize pharmacological tools developed for mammals to investigate zebrafish brain and behavior function. However, the bottleneck for zebrafish research has been the paucity of appropriate screening tools, e.g. behavioral paradigms that would allow proper detection of phenotypical alterations of brain function induced by novel compounds and/or mutations.

Methods: Here we present a new learning task for zebrafish, a plus maze paradigm in which zebrafish are required to acquire and remember the association between a visual stimulus (CS) and the presentation of a shoal (US, a group of fish, which is a rewarding stimulus). Subsequent to the completion of training, we test the response of zebrafish to the CS (visual cue) alone at a probe trial. We analyze the performance of two groups of zebrafish: a paired group for which the US and CS are presented together, and an unpaired group for which the US and CS are presented in random spatial locations. Furthermore, we examine the effect of the NMDA-R antagonist MK801 on motor function and visual and lateral line perception as well as on learning and memory performance in this novel task. Using a between subject design, we administer MK801 before training (drug on board during acquisition), after training (drug on board during consolidation of memory), and before the probe trial (drug on board during recall of memory).

Results: We demonstrate that zebrafish exhibit an excellent learning performance in the plus maze associative learning task as revealed by the significant preference for staying in the proximity of the CS shown by fish of the paired but not the non-paired group. We also show that MK801 administration significantly disrupts learning and memory performance in the plus maze at a dose that we demonstrate does not alter such performance characteristics as motor function and/or perception. Importantly, we find that the disruption occurs if the drug is administered right after, but not before, the training, or if it is given right before the probe trial suggesting that MK801 can disrupt consolidation of memory and can also impair recall of memory but does not alter acquisition processes in the plus maze learning task in zebrafish.

Discussion: We argue that the similarities between the zebrafish plus maze associative learning task and rodent radial arm maze tasks represent good face validity. We also claim that the finding of NMDA-R antagonist induced learning and memory performance deficits represent good construct and predictive validity of this task. In summary, we propose that zebrafish may have an excellent future in psychopharmacological and behavior genetic analysis of vertebrate learning and memory.

Disclosure: M. Sison: None. R. Gerlai: None.

6. Adolescent Cannabinoid Administration Leads to Long Term Molecular and Behavioral Deficits in Mice.

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Background: Cannabis is the most commonly abused illicit drug in the United States. 2.1 million Americans used cannabis for the first time in 2007; of them 62.2% were less than 18 years old. In the 12-17 year age group, there has been a dramatic rise from 1.8% to 21.9% (between 1965 to 2001) in the number of individuals who report ever using cannabis. This is of particular health concern given the large body of literature that shows an association between adolescent cannabis use and adult onset of psychosis. A recent systematic review of longitudinal studies of cannabis use and subsequent psychotic outcomes reported a 40% increased risk of psychotic outcome in individuals who had ever used

cannabis. Schizophrenia did not develop days or weeks after cannabis use, but years later, suggesting that cannabis use during a critical period of brain maturation may lead to long-term effects. These human studies demonstrate associations but do not demonstrate causality.

Methods: We conducted a series of experiments to examine the cause-effect relationship between cannabis and schizophrenia in which we administered cannabinoids to mice at different ages and conducted molecular and behavioral assays once these mice were adults. Mice were administered with a cannabinoid receptor 1 (CB1) agonist, (WIN 55,212-2), CB1 antagonist (AM 251), both agonist and antagonist or vehicle for 10 days by I.P. injection at different developmental time points (5, 7 and 9 weeks). At 16 weeks of age, behavioral tests were carried out followed by molecular studies 1 week after the last behavioral test.

Results: Mice administered WIN 55,212-2 (WIN) at 5 weeks of age display specific schizophrenia-like behaviors, specifically deficits in prepulse inhibition and deficits in a learning and memory paradigm. These behavioral deficits were not observed in mice treated with the CB1 agonist at later developmental time points. Group I metabotropic glutamate receptor (mGluR) activation drives endocannabinoid synthesis and release. In our model mice, we find significant increases in CB1 (control 0.45 ± 0.09 ; WIN 0.63 ± 0.11 units) and mGluR5 (control 0.54 ± 0.67 ; WIN 2.07 ± 0.47 units) receptor protein while mGluR1a levels are significantly reduced (control 2.31 ± 0.73 ; WIN 1.03 ± 0.32 units). Both CB1 and mGluR5 protein levels are significantly correlate with learning and memory deficits ($r = -0.82$, $p = 0.003$ and $r = -0.67$, $p = 0.03$ respectively). These data suggest that the administration of exogenous cannabinoids leads to sustained upregulation in the brain's endogenous endocannabinoid (eCB) system that is associated with behavioral sequelae.

Discussion: These data show that cannabinoid administration leads to long term molecular and behavioral changes in an age-dependent manner. Our model mice closely resemble the human condition in that early (adolescent), but not adult, cannabinoid exposure leads to schizophrenia-like behaviors. This may provide a model animal in which to test the mechanisms by which adolescent cannabinoid exposure leads to molecular changes predisposing to schizophrenia.

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7. Post-Weaning Chronic Social Isolation Produces Significant Behavioral Dysregulation with Decreases in Prefrontal Cortex Synaptic-Associated Protein and Glutamate Receptor Expression in Female Rats

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Background: Early life stressors in rodents, including maternal separation and social isolation, have been widely shown to disrupt brain development and profoundly affect a wide-range of adult behaviors. In this study, we focus on the development of female Sprague-Dawley rats in the presence and absence of conspecifics across the critical period of social play through young adulthood. Similar male rodent models have shown that this form of social deprivation results in dysregulated dopaminergic and serotonergic function with core features of a few neuropsychiatric disorders including anxiety disorder and schizophrenia (Fone, 2008). Here we examine the behavioral and biochemical effects of social isolation in female rats. Our findings include the effects of this form of social deprivation on a suite of behavioral measures and on the expression of synaptic-associated proteins and glutamate receptors in the prefrontal cortex (PFC).

Methods: Female Sprague-Dawley rats were weaned at post natal day (PND) 18 and placed in either social housing with two or more animals per cage ($N = 8$) or isolation housing ($N = 8$) for a period of ten weeks. The isolated rats have sight, sound and smell of conspecifics in the

shared colony room but are without touch and the affiliative context of group housing. Animal use procedures were in accordance with the Yale University Care and Use of laboratory animals' guidelines. Behavioral effects of chronic social isolation housing were measured using a suite of well-established behavioral assessments including open field test (OFT), novelty suppressed feeding test (NSFT), social interaction test (SIT) and sucrose preference test (SPT). Additional measures of physiological function, including weight and ovarian cyclicity, are included. At the end of behavioral testing, animals were sacrificed and biochemical measures of prefrontal cortex (PFC) function were obtained. These measures included Western Blot analysis of synaptic-associated proteins, including post-synaptic marker PSD95 and pre-synaptic marker Synapsin 1, and glutamate receptors, including AMPA receptor subunit GLUR1 and NMDA receptor subunit NR1. Data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC). For all experiments except the novelty-suppressed feeding tests, student T-tests were performed. The significance level was set at 0.05. In the novelty-suppressed feeding test, we used the Kaplan-Meier survival analysis because of the lack of normal distribution of the data.

Results: Behavior: Social isolation produced hyperactivity in the OFT and anxiogenic effects in NSFT. Socially isolated animals spent significantly less time with conspecifics in the SIT. Physiology: Socially isolated females were significantly heavier than group housed isolated animals at the end of the experiment. To avoid potential confounding effects of sex steroids on brain biochemistry assays, measures of ovarian cyclicity were obtained prior to sacrifice. State of estrus was found to be random with respect to housing. Biochemical Measures: Socially isolated females showed markedly lower levels of synaptic-associated proteins, PSD95 and Synapsin 1 as well as glutamate receptors GLUR1 and NR1 expression as compared to group housed animals.

Discussion: Results of this study contribute additional insights into the neurodevelopmental consequences of early life stress and adversity. Specifically, sustained early life exposure to social isolation for female rats had significant behavioral and neurochemical consequences including the ability to cope with novelty, species-specific patterns of sociality, and synaptogenesis and glutamate functions. This anxious/depressed phenotype with impairments in PFC neurochemistry has relevance for a host of neuropsychiatric disorders.

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8. Infant Behavior and Central Serotonin are Altered in Adopted Infants: A Nonhuman Primate Model

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Background: Human studies show that adoptees are at risk for a number of psychopathological behaviors. While there has been some suggestion that this increased risk for psychopathology in adoptees is due to an increased genetic loading in parents who are unable to care for their offspring, studies investigating early treatment of adopted infants are sparse, and other studies suggest that early mother-infant conflict is a risk factor for psychopathological behavior and pathological CNS development. To understand the etiology of risk for psychopathology in adoptees, we used a nonhuman primate model to assess behavior and CNS serotonin functioning in infants reared by their biological or an adopted mother.

Methods: 150 socially housed rhesus macaques were studied, including 107 infants reared with their biological mothers and 43 infants reared with their unrelated adoptive mothers. Adopted infants were randomly assigned to unrelated adoptive mothers based on their date of birth and the adoptive mother's date of giving birth. Infants were removed from their biological mothers within 72 hours of birth and placed with

an unrelated, lactating adoptive mother who had also recently given birth. Adopted mothers showed no apparent indication that could tell the difference between the adopted infant and their biological infant that had been removed shortly before the adoption. Twenty mother-infant behaviors representing maternal treatment, infant anxiety and aggression were recorded across the first 6 months of life. Cerebrospinal fluid was obtained monthly over the first 6 months of life and assayed for levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). Blood was sampled for levels of ACTH and cortisol.

Results: No mother showed evidence of abuse or neglect. When compared to the infants reared by their biological mothers, adopted infants were restrained less by their mothers, approached and left their mothers more frequently, spent more time away from their mother exploring the environment and in independent activity. Adopted infants also were less likely to be held by their mother and received more maternal rejections than infants reared by their biological mother, indicating the adopted infant was more responsible for maintaining the mother-infant relationship than was the adopted mother. Perhaps as result, they exhibited more anxiety-like self-directed behaviors and exhibited significantly higher plasma ACTH during stress. They also directed and received more aggression from other social group members. There was evidence that the treatment differences by adopted mothers and other group members altered central serotonin functioning. Adopted infants exhibited significantly lower CSF 5-HIAA when compared to infants reared by their biological mothers, particularly during the early months of life.

Discussion: To the extent that our data generalize to humans, the data suggests greater risk for anxiety and aggression in adopted infants, accompanied with impaired CNS serotonin functioning. Impaired central serotonin functioning has been implicated in a number of psychopathological disorders and may be in part what places adoptees at risk. The difficulties found in adopted infants may be explained by what Chess and Thomas called a "goodness of fit" between parental temperament and infant temperament. The infant's temperament and mother's personality may fit in a glove-like fashion or it can be conflictual, ultimately determining the "goodness of fit" of the parent-infant relationship (Chess & Thomas, 1991). According to Chess and Thomas when mother and infant temperament do not match, this can lead to a more arduous relationship. While somewhat speculative, one possible explanation for our findings are that adopted mothers and their unrelated infants share less genetic commonalities than infants who are reared by their biological mother and as a consequence are less likely to share common temperaments.

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9. Up-Regulation of Anandamide Hydrolysis Following Chronic Stress Drives Changes in Amygdala Morphology and Emotional Behavior

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Background: Endocannabinoid signalling is known to modulate anxiety-like behaviours. In particular, inhibition of fatty acid amide hydrolase (FAAH), the enzyme responsible for the metabolism of the endocannabinoid ligand anandamide (AEA), produces anxiolytic effects in an array of preclinical models. Both FAAH activity and AEA content are known to be modulated by stressful stimuli in brain regions, such as the amygdala, which are critical for the generation of anxiety and fear.

Methods: The current study aimed to examine the role of endocannabinoid signalling in the development of anxiety and morphological changes within the amygdala following chronic stress.

Results: Exposure of mice to 21 days of 6 h/day restraint stress resulted in a significant reduction in AEA content within the amygdala, but did

not modulate amygdalar levels of the other endocannabinoid ligand 2-arachidonoylglycerol, or the binding site density of the cannabinoid CB₁ receptor. This reduction in AEA content was met by a significant increase in the constitutive hydrolytic activity of FAAH within the amygdala, indicating that chronic stress results in an increase in basal FAAH-mediated AEA hydrolysis. To determine if this increase in FAAH and loss of AEA signaling was involved in changes in amygdalar morphology and emotional behaviour we examined the effects of chronic stress in wild type and FAAH deficient mice on anxiety-like behaviour and amygdalar morphology. Wildtype mice exhibited an increase in anxiety-like behaviour in the elevated plus maze and an increase in dendritic spine formation on branches from pyramidal neurons in the basolateral amygdala. Mice lacking FAAH were resistant to both changes in anxiety-like behaviour and spinogenesis in the basolateral amygdala.

Discussion: These data suggest that chronic stress results in an activation of FAAH-mediated AEA hydrolysis within the amygdala, which in turn contributes to the development of anxiety-like behaviours and changes in amygdalar morphology. Collectively, these data also support the hypothesis that inhibition of FAAH may be a suitable target for the development of anxiolytic agents.

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10. NudE-like 1 (NDEL1) in nNOS Signaling for Cortical Development and NO-Mediated Behaviors

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Background: Higher brain function and behavior are influenced by neuronal circuit formation during brain development. Many genetic risk factors for schizophrenia, such as Disrupted-in-Schizophrenia-1 (DISC1) and neuronal nitric oxide synthase (nNOS), have key roles in neurodevelopment. Consequently, disturbances in brain development are suggested to underlie the pathology of such devastating condition. Although roles for these factors have been reported at the molecular level, there are limited studies on whether they act in common molecular pathways, contributing to disease pathology. In this study, we will explore the role of NudE-like 1 (NDEL1), a schizophrenia-associated protein interactor of DISC1, in nNOS signaling for the development of prefrontal cortex and resultant behaviors. nNOS signaling regulates neuronal differentiation, leading to proper neuronal circuit formation. As a result, mice with genetic deletion of nNOS display diverse behavioral deficits, such as aggression and impaired spatial memory. However, prefrontal cortex-associated behaviors in nNOS knockout (KO) mice have not been studied yet, whereas prefrontal cortex-associated cognitive deficits have been frequently reported in schizophrenia. We have previously reported that DISC1-NDEL1 interaction regulates neurite outgrowth. Interestingly, NDEL1 regulates the activity of Cdc42, a critical regulator for dendritic development. Given that nNOS, NDEL1, and DISC1 are highly expressed in cortical plate in developing cerebral cortex, NDEL1 may function as a downstream effector of nNOS signaling regulated by DISC1 for dendritic development, which may contribute to NO-mediated establishment of neuronal circuits responsible for long-lasting behaviors.

Methods: We examine the role of S-nitrosylation of NDEL1 via nNOS signaling for dendritic development and their underlying molecular mechanisms by using cortical neuron cultures with RNAi approaches and biochemical experiments with brains from nNOS KO mice. We also examine whether genetic deletion of nNOS results in impaired dendritic development in prefrontal cortex. By using *in utero* inducible gene expression system, overexpression of mutant NDEL1 deficient in DISC1 binding in post-migratory neurons is tested for compensation for dendritic abnormalities in nNOS KO mice. Behavioral

characterization of nNOS KO mice and the impact of mutant NDEL1 on behavioral phenotypes are also examined.

Results: We found that NDEL1 is S-nitrosylated in the brain of wild-type mice, but not of nNOS knockout mice. We also confirmed that nNOS-DISC1 and nNOS-NDEL1 interaction in the developing cerebral cortex. The binding of nNOS with NDEL1 is reduced in DISC1 KO mice, indicating that DISC1 is required for the nNOS-NDEL1 interaction. Furthermore, our results from behavioral characterization of nNOS KO mice suggest that prefrontal cortex-mediated cognitive functions may be impaired in nNOS KO mice.

Discussion: NOS signaling has been recently identified as a possible disease pathway for schizophrenia by the analysis of rare structural variants. Nonetheless, the underlying mechanisms by which nNOS signaling contribute to neuronal circuit formation and disease pathology are still unknown. In this study, we propose the possible convergent signaling between nNOS, NDEL1, and DISC1, three genetic risk factors for schizophrenia, for the establishment of neuronal circuits which are required for high brain functions, especially prefrontal cortex-mediated behaviors. We will use an innovative approach that manipulates NDEL1 function in specific cell behavior and evaluate its effect on resultant behaviors in nNOS KO mice, based on the technique we have recently established. Our results will provide us with important clues for the possible involvement of nNOS/DISC1/NDEL1 signaling in the etiology of schizophrenia.

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11. Prenatal Stress Potentiation of Cocaine-Seeking is Dependent on Genetic Background in Mice

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Background: Cocaine addiction is mediated by a complex phenotype resulting from gene-environment interactions. There is substantial support that genetic background contributes to individual differences in the psychostimulant and rewarding properties of cocaine. There is also substantial evidence that stressful experience during the prenatal period or early postnatal period alters the developmental trajectory of the nervous system, resulting in a complex array of abnormal behavioral features, including enhanced responsiveness to cocaine and other drugs of abuse.

Methods: To investigate the interaction between genetic background and early environmental insults on cocaine-seeking behavior, we determined the effect of prenatal stress (PNS) in two genetically-distinct, inbred strains of mice (C57BL/6J and DBA/2J) on conditioned place preference (CPP). Pregnant dams were subjected to repeated restraint stress (1h, 3 x day) from E14 until birth (PNS) or left undisturbed (control) and cocaine-induced CPP was assessed in the offspring during adulthood. CPP was induced by repeated pairings of cocaine exposure (4 x 10 mg/kg, i.p.) with one of two distinctive compartments of the test apparatus and repeated pairings of vehicle (saline) exposure (4 x 10 mg/kg, i.p.) with the other compartment. Then, each mouse was allowed to move freely between the two compartments and the amount of time spent in the cocaine-paired compartment versus the saline-paired compartment indexed CPP.

Results: PNS significantly increased the magnitude of the CPP in B6 mice whereas there was a nonsignificant decrease in the magnitude of CPP in D mice. Conversely, D2, relative to B6, mice exhibited increased psychostimulant response following each cocaine injection, greater sensitization in the psychostimulant response, and increased conditioned locomotion (during the test for place conditioning), however, none of these measures were significantly impacted by PNS.

Discussion: The results of the present study indicate that genetic background interacts with PNS to determine cocaine-seeking behavior in adult mice, thus, PNS and mouse strains offers a viable avenue

to elucidate the details of the impact of gene-environment interactions on adult drug-seeking and, potentially, elements of addiction vulnerability. Supported by National Alliance for Research on Schizophrenia and Affective Disorders Young Investigator Awards to TEK and KKS, NIDA (1DA027115-01) to TEK, and NIDA (1DA024038-01) to KKS.

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12. The Effects of Simultaneous Cocaine and Alcohol Consumption on Glutamatergic Markers in the Nucleus Accumbens of the Rat Lori Knackstedt*

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Background: Cocaine addiction remains a substantial public health problem in the United States today. It is widely recognized that a high risk of relapse exists even after long periods of abstinence and this relapse represents one of the key challenges in the successful treatment of cocaine addiction. While many pharmacological agents have shown efficacy at blocking the reinstatement of cocaine-seeking in this animal model of relapse, none of these agents have shown to be effective at preventing relapse in humans and no FDA-approved drug exists for cocaine addiction. One explanation for the failure of these agents to translate well into the clinic could lie in the fact that most cocaine addicts are poly-substance users, in particular using alcohol with cocaine. It has been estimated that up to 90% of cocaine addicts simultaneously use alcohol. It is possible that the combination of alcohol and cocaine produce different neuroadaptations than either drug alone. I have developed a rodent model of alcohol and cocaine co-abuse in which animals self-administer alcohol for 6 hr/day following a 2 hr operant cocaine self-administration session.

Methods: Animals are trained to drink ethanol (20% v/v) using the Intermittent-access Drinking Paradigm (IDP), wherein animals are given unsweetened 20% ethanol in the home cage for 24 hr on alternating days without water deprivation. Following 6 sessions of IDP, animals were trained to self-administer cocaine in the operant chamber and were permitted access to 20% alcohol in the home-cage for 6 hours following their self-administration session. Operant cocaine self-administration continued for 12 days before animals were placed into extinction training. Western blotting will be conducted in order to assess nucleus accumbens glutamatergic neuroadaptations following the self-administration of cocaine and alcohol alone and in combination relative to saline controls.

Results: Post-cocaine access to oral alcohol produced a significant increase in the amount of cocaine self-administered during the first days of operant cocaine self-administration in comparison to animals not permitted to drink alcohol following cocaine self-administration. However, cocaine self-administration did not increase alcohol consumption; cocaine self-administering and yoked-saline controls demonstrated identical levels of alcohol consumption.

Discussion: These results indicate that alcohol self-administration can modulate the amount of cocaine self-administered by rats and shed light on glutamatergic adaptations in the nucleus accumbens following self-administration of cocaine and alcohol.

Disclosure: L. Knackstedt: None.

13. A Rodent Model of Schizophrenia-Related GAD67 Deficiency Exhibits Deficits in Both Synaptic Transmission and Behavioral Phenotype in Juveniles, with Recovery in Adulthood Matthew Lazarus*, Z. Josh Huang

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Background: A highly reproducible molecular pathology in schizophrenia (SZ) is the reduction of mRNA for GAD67, the principle enzyme for GABA synthesis in neocortex, in certain types of inhibitory

interneurons, particularly those expressing parvalbumin (PV). GAD67 expression is regulated by neural activity, and its reduction in schizophrenia is mostly likely a downstream impact of reduced engagement of the PV cell network. Because PV cells play an important role in the generation and maintenance of gamma wave activity, dysfunction in these cells is hypothesized to contribute to cognitive deficits in SZ, such as working memory impairment. However, the impact of GAD67 deficiency on synaptic transmission, network properties, and behavior has not been tested. At present, there are no effective treatments to target GABA-system dysfunction in schizophrenia, or to significantly limit cognitive deficits in the disease.

Methods: To model the cell-type specific reduction of GAD67 observed in SZ, we used a cre/lox system in transgenic mice to conditionally knockdown Gad1, which encodes GAD67, in PV cells. Both young mice (~4 weeks old) and adult mice (>8 weeks old) were studied. Viral-vector mediated GFP expression was used to identify PV cells for electrophysiological analysis, particularly paired recordings from PV to pyramidal cells. Behavioral tests, including Y-maze spontaneous alternation to assess working memory, were performed.

Results: In young mice, reduction of GAD67 in PV cells led to weakened synaptic inhibition. Removal of one allele of Gad1 (heterozygote) led to a 33% reduction of GAD67 in PV cells ($P < 0.001$). This caused a supralinear deficit in synaptic transmission: Unitary inhibitory post-synaptic current (uIPSC) charge transfer was decreased by 65% ($P < 0.01$), the result of both reduced uIPSC amplitude (57%; $P < 0.05$) and faster uIPSC decay time (20%; $P < 0.01$). Additionally, pyramidal cells showed hyperactive spiking properties in response to current injection (Spike frequency increased 29%; $P < 0.05$), likely a direct consequence of reduced inhibitory control. Furthermore, young mutant mice demonstrated a behavioral phenotype, with reduced spontaneous alternation in the Y-maze (correct choice reduced 19%; $P = 0.08$ for synaptic and behavioral properties). These physiological and behavioral recoveries likely result from compensatory mechanisms at the molecular and circuitry levels.

Discussion: In schizophrenia, environmental insults and genetic predisposition may disrupt cortical circuits, leading to reduced engagement of PV cell activity and sustained reduction of GAD67 expression in these cells. Here, we have directly modeled this endophenotype of GAD67 deficiency in mice, without altering upstream activity. We have demonstrated for the first time that GAD67 deficiency does in fact lead to significantly weakened synaptic transmission in PFC, with a concurrent behavioral phenotype. However, weakened inhibition in juvenile mice appears transient and does not produce any irreversible changes. Homeostatic mechanisms appear able to compensate for the impact of GAD67 deficiency (i.e. pyramidal cell hyperactivity may lead to increased excitation of PV cells, and therefore upregulation of Gad1 transcription). Our results suggest that PV cell function, or possibly GAD67 expression level itself, presents a potential treatment target for cognitive dysfunction, which may provide therapeutic benefit even in adult schizophrenia patients.

Disclosure: M. Lazarus: None. Z. Huang: None.

14. mTOR-Dependent Synaptic Protein Synthesis and Synapse Formation Underlie the Rapid Antidepressant Actions of Ketamine Nanxin Li*, Boyoung Lee, Rong-Jian Liu, Mounira Banasr, Jason Dwyer, Masaaki Iwata, Xiao-Yuan Li, George Aghajanian, Ronald Duman

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Background: Major depressive disorder (MDD) is one of the most prevalent and debilitating illnesses world wide, affecting ~17 percent of the population and causing enormous personal and economic burden. The impact of MDD is underscored by the limitations of currently available medications, including low response rates,

treatment resistant patients, and a time-lag of weeks to months for a therapeutic effect. These data highlight a major unmet need for more efficacious and faster-acting antidepressant agents. Recent clinical studies demonstrate that a single low dose of the drug ketamine, an antagonist of the excitatory neurotransmitter glutamate-NMDA receptor, produces rapid antidepressant actions (2 hr) that last for up to 7 days in treatment resistant patients. This rapid action, by a mechanism completely different from typical monoamine reuptake inhibitors, represents one of the most significant findings in the field of depression over the past 2 decades. However, the mechanisms underlying the actions of ketamine are more complicated than simple NMDA receptor blockade. In addition, ketamine is a psychotomimetic agent and has abuse potential, limiting widespread and repeated therapeutic administration, stressing the need for safer drugs that can be administered repeatedly and/or that have long-lasting effects.

Methods: We carried out a series of studies to examine the cellular signaling pathways that mediate the behavioral actions of ketamine, focusing on signaling cascades known to rapidly influence synaptic plasticity. We used a brain tissue preparation method enriched in synaptoneurosomes and western blotting methods to measure expression levels of several protein kinases. Two-photon confocal imaging was used to study the spine number and morphology of prefrontal cortex (PFC) pyramidal neurons. Several rodent models of depression, including forced swim test (FST), learned helplessness (LH), novelty suppressed feeding test (NSFT), and chronic unpredictable stress (CUS) were used to probe the behavioral actions of ketamine and related signaling pathways.

Results: We have identified a novel action of ketamine, rapid stimulation of the mammalian target of rapamycin (mTOR) in rat PFC. mTOR is a protein kinase that integrates cellular signaling and coordinates the control of translation, a process by which new proteins are synthesized to meet cellular demands. Interestingly, mTOR and associated translational machinery are localized to dendrites and spines of neurons and have been linked to regulation of protein synthesis-dependent long-term synaptic plasticity in cellular and behavioral models. A role for protein synthesis-dependent plasticity in the antidepressant actions of NMDA receptor blockade is supported by our studies demonstrating that ketamine rapidly increases levels of synaptic proteins (synapsin I, PSD95, GluR1) and increases synaptogenesis (number and function of spines) of pyramidal neurons in PFC. We have also found that ketamine administration produces rapid antidepressant responses in rodent behavioral models (FST, LH, NSFT). Moreover, the ketamine-induction of synaptic proteins, synaptogenesis, and rapid antidepressant actions are blocked by rapamycin, demonstrating a requirement for stimulation of mTOR signaling. These effects of NMDA receptor blockade are particularly important in light of reports that depression and stress are associated with atrophy of PFC. Brain imaging studies have reported that the volume or size of the PFC is decreased in depressed subjects, and basic research studies demonstrate that repeated stress causes atrophy of neurons in the PFC. By rapidly increasing synaptogenesis ketamine could oppose or reverse the damaging effects of depression and stress. In support of this hypothesis, we demonstrate that ketamine rapidly reverses the deficit in synaptic proteins and behavioral abnormalities produced by exposure to CUS. The current findings are particularly notable, showing that ketamine can rapidly reverse both the structural and behavioral deficits that occur over the course of chronic stress (3 weeks) exposure.

Discussion: Together, the discovery that increased synaptogenesis and connectivity in PFC underlie the rapid antidepressant actions of ketamine represents a fundamental shift in our understanding of the treatment of depression, and will lead to the identification of novel targets for the treatment of MDD.

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15. Pivotal Role of RGS2 Gene Expression in Anxiety and Depression; Relationship to Serotonin 1A and 1B Receptor Function Tzuri Lifschytz*, Esther Channa Broner, Lior Greenbaum, Bernard Lerer

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Background: Regulators of G protein signaling (RGS) negatively modulate G protein receptors by promoting GTP hydrolysis. Molecular variants of the RGS2 gene have been associated with anxiety and depression in humans but a mechanism has not been demonstrated. We studied the relationship of level of expression of RGS2 to behaviors putatively reflecting anxiety and/or depression and to 5-HT_{1A/1B} receptor expression and function in genetically modified mice.

Methods: Male C57BL mice, wildtype (WT), heterozygous (HET) or homozygous (HOM) for an RGS2 knockout (KO) mutation, underwent the novelty suppressed feeding (NSFT), elevated plus maze (EPM) and forced swim tests (FST). We measured 5-HT_{1A/1B} receptor expression in the cortex and raphe by real time PCR and 5-HT_{1A} receptor function by 8-OH-DPAT induced hypothermia.

Results: Significant RGS2 genotype effects (HOM > HET ≥ WT) were found in the NSFT (latency to feed, $p = 0.001$), EPM (time spent in closed arms, $p = 0.0006$), and FST (immobility, $p = 0.035$). There was a direct relationship between RGS2 and 5-HT_{1A/1B} receptor expression in the raphe (HOM ≤ HET < WT; $p < 0.0001$). In the cortex (where 5-HT_{1A} receptors are post-synaptic) the relationship was inverse (WT > HET ≥ HOM; $p = 0.06$). 8-OH-DPAT induced hypothermia was significantly diminished in RGS2-HET/HOM mice ($p = 0.005$).

Discussion: Our findings indicate that lower RGS2 expression is associated with greater levels of anxiety/depression. Lower 5-HT_{1A/1B} receptor expression in the raphe of RGS2-HET/HOM mice is attributable to compensatory down-regulation due to chronic over-activity of the receptor. This finding is corroborated by the reduced 8-OH-DPAT induced hypothermia observed in RGS2-HET/HOM mice. Conversely, greater cortical 5-HT_{1A} expression in RGS2-HOM mice may be a compensatory response to lower serotonin availability (due to greater activity of inhibitory raphe 5-HT_{1A/1B} receptors). These findings have potentially important implications for treatment development and personalization in anxiety and depressive disorders in that RGS2 expression level (which may be genetically influenced) may impact upon basal affective state and possibly response to treatment. [Supported in part by grants from the National Institute for Psychobiology in Israel (to TL) and the Institute for the Study of Affective Neuroscience (to BL)].

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16. MDMA (ecstasy) Regulates Activity and Genes Implicated in Neurodevelopment Differently in Brain of Adolescent and Adult Mice Bertha Madras*, Eric Vallender, Gregory A. Miller, Brad Constant

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Background: The adolescent brain is particularly susceptible to addiction. Risk analyses has shown that early onset of drug use, in contrast with adult onset of use, confers a much higher risk for developing an addiction to alcohol, nicotine, amphetamines, cocaine, inhalants, prescription opioids, anxiolytics, and other drugs. We focus on MDMA (3, 4-methylenedioxymethamphetamine, ecstasy) because: (1) it is one of a few drugs displaying increased use among 10th graders, and declining perception of "great risk" for users among 8th, 10th and 12th graders; (2). frequent MDMA users exhibit a reduction in markers for serotonin neurons, selective impairment of working and episodic memory, sleep disorders, depression, and residual cognitive deficits, which may not resolve with prolonged abstinence; (3) among hallucinogens, the risks for MDMA addiction are high with early onset of use and are associated with reduced academic achievement and abuse/addiction to other drugs; (5) Seven

out of ten recent MDMA users report experiencing an SUD in the past year. To clarify heightened vulnerability to MDMA consequences in adolescents, we hypothesize that introduction of drugs during neurodevelopment may alter the trajectory of normal neurodevelopment. Specifically, we proposed that MDMA regulates axonal guidance molecules (AGMs), critical for neurodevelopment, neurogenesis and neuroadaptation, differently in the adolescent brain than in the adult brain. We interrogated this hypothesis by comparing the response of adolescent and the adult mice to repeated exposure to MDMA.

Methods: 12 adolescent male and female mice (23-33 days) and 12 adult mice (10 weeks) were used in this study. Mice were administered a relatively low dose of MDMA (3mg/kg) daily for 6 days (with a 2-day interval), with controls receiving saline. During this period locomotor activity and stereotypy were measured. Following euthanasia, real-time PCR was used to measure mRNA expression of 24 genes implicated in MDMA effects. First, we demonstrated the feasibility of detecting AGMs in mouse brain. Then, we compared AGM mRNA expression in hippocampus and cerebellum of each subject treated with saline or MDMA.

Results: Initially, adolescent mice displayed lower levels of activity than adult mice. MDMA did not alter weight gain in the two cohorts of mice but had different effects on adolescent and adult mice: (1) it depressed locomotor activity in adolescent mice but increased activity in adult mice; (2) in adolescent mice, the onset of MDMA changes in locomotion occurred at earlier times and waned faster than in adults. Real-time quantitative PCR revealed robust mRNA expression of Ephs (receptors) and ephrins (ligands), semaphorins, DCC, netrin 1, reelin, neuropilin-1, *dus6P*, *UNC5A* and *D1*, *D2*, *D4*, *D5* dopamine receptors. In hippocampus, several genes were altered differently in adolescent than in adult tissue. In cerebellum, genes were not only altered differently in adolescent and adult mice, but striking male/female differences in response to MDMA were observed.

Discussion: This pilot study demonstrates that MDMA: (1) altered locomotor activity differently in adolescent and adult mice, both in the direction of change and time course; (2) altered expression levels of specific AGMs in hippocampus and cerebellum, conceivably linking the cascade of MDMA-induced neurodevelopmental, toxicological, morphological, behavioral, cognitive changes in hippocampus to function of axonal guidance molecules. Combined with other strategies, this novel approach may clarify the role of AGMs in mediating the adverse consequences of METH and similar psychostimulant drugs of abuse.

Disclosure: B. Madras: Part 1; Alseres, Organix. E. Vallender: None. G. Miller: None. B. Constant: None.

17. Ethanol Exposure in Adolescent Rats Results in Diminished Attentional Deficit and Increased Impulsivity after Ethanol Administration in Adulthood

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Background: Many people begin alcohol use during adolescence. Adolescence is a critical period for brain development that is particularly vulnerable to the neurotoxic effects of alcohol. Adults who began alcohol drinking during adolescence exhibit impaired attention and increased impulsivity. However, human studies cannot distinguish whether increased impulsivity and impaired attention are pre-existing conditions or the result of drinking during adolescence. Here, we examined the long-term effects of adolescent intermittent ethanol (AIE) exposure on rats' performance in the 5-choice serial reaction time task (5-CSRTT) during adulthood. The 5-CSRTT is a cognitive task that allows simultaneous assessment of attention (accuracy, correct responses, omissions), impulsivity (premature responses), and cognitive flexibility (time-out responses). We examined performance in this task in AIE- and vehicle-exposed rats: (i) under baseline conditions; (ii) after challenge conditions that increased cognitive load; and (iii) after acute ethanol (EtOH) administration.

Methods: Adolescent male Wistar rats arrived in the lab on postnatal day 27 (P27). Rats were exposed to a 4-day EtOH binge that involved

the administration of 5 g/kg of 25% w/v EtOH every 8 h resulting in a total of 12 intragastric administrations during adolescence (P33-36, n=12). Control rats (n=12) received sterile water on a similar schedule. On P60, training in the 5-CSRTT was initiated. Approximately 40 sessions were required for the rats to reach asymptotic performance, at which time rats were approximately at P100. After stable baseline performance was achieved, rats were exposed to several task challenges. Specifically, stimulus duration was decreased from 1 s to 0.5 or 0.25 s to assess the magnitude of attentional performance disruption; or the inter-trial interval was increased from 5 s to 15 s to assess changes in impulsive behavior. Finally, rats were challenged acutely in a random order with either of two EtOH doses (1.5 and 3 g/kg, 25% w/v EtOH, IG) or vehicle 5 min before testing in the 5-CSRTT. These task challenges occurred once a week.

Results: AIE exposure produced high blood EtOH levels (229.74 ± 18.43 mg/dl on the last day of the EtOH binge). During adulthood, AIE-exposed rats exhibited only small deficits in task acquisition and baseline performance in the 5-CSRTT. Specifically, a larger percentage (50%) of AIE-exposed rats were slow in meeting performance criteria during the early stages of training compared to control rats (25%), and exhibited some cognitive inflexibility reflected in higher number of time-out responses than vehicle-treated rats. During challenge with a short duration stimulus, performance of both AIE- and vehicle-exposed rats deteriorated. However, AIE-exposed rats made more correct responses and fewer omissions than control rats, indicating lesser disruption of attentional performance of AIE-exposed rats compared to controls. The challenge with the long inter-trial interval resulted in increased premature responses in both AIE- and vehicle-exposed rats, with no differences between the two groups. The highest dose of acute EtOH decreased the number of trials completed and the percentage of correct responses and increased the percentage of omissions in control rats, indicating disruptive effects of EtOH on attention. Interestingly, this EtOH challenge had no effect on AIE-exposed rats' performance, indicating tolerance to the disruptive effects of acute EtOH. Further, AIE-exposed, but not control, rats exhibited increased premature responses after either EtOH or vehicle challenge, indicating increased impulsivity.

Discussion: AIE-exposed rats tested in adulthood exhibited only small learning deficits and cognitive inflexibility when tested under baseline conditions. When cognitive demands were increased by implementing task challenges, the performance of AIE rats was less affected than that of control rats, suggesting improved ability to deal with mildly challenging cognitive conditions. In addition, attentional performance of AIE rats was less disrupted by acute EtOH administration, reflecting tolerance to the disruptive effects of EtOH that may lead to increased drinking in adulthood, and thus dependence. Finally, AIE-exposed, but not control, rats exhibited increased impulsive action after acute EtOH or vehicle challenge, another effect that may lead to increased alcohol drinking and other maladaptive behaviors in adulthood. In conclusion, EtOH binge exposure during adolescence led to phenotypes and responses to alcohol in adulthood that may lead to increased propensity to alcohol dependence.

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18. Specific Role of VTA Dopamine Neuronal Firing Rates and Morphology in the Reversal of Anxiety-Related, but not Depression-Related Behavior in the *ClockΔ19* Mouse Model of Mania

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Background: Bipolar disorder is a chronic psychiatric disease that is characterized by bouts of both depression and mania. A wealth of

clinical and pre-clinical studies have suggested an important role for both circadian rhythm disruption and dopaminergic transmission in bipolar disorder. However, the exact role for dopamine in this disease, and the interaction between the circadian system and dopaminergic pathways remain unclear. Our previous studies found that mice with a mutation in the *Clock* gene (*Clock* Δ 19) have a complete behavioral profile that is very similar to human mania which can be reversed with chronic lithium treatment.

Methods: We utilized immunohistochemistry and measurements of neuronal morphology, patch clamp electrophysiology, viral mediated gene transfer, and a battery of behavioral measures.

Results: Here we find that *Clock* Δ 19 mice have an increase in dopaminergic activity in the ventral tegmental area (VTA), and that chronic lithium treatment selectively reduces the firing rate in the mutant mice with no effect on dopamine cell activity in wild type mice. The increased dopaminergic activity in the *Clock* mutants is associated with cell volume changes in dopamine neurons which are also rescued by lithium treatment. To determine the role of dopaminergic activity and morphological changes in dopamine neurons in manic-like behavior, we manipulated the excitability of these neurons by overexpressing an inwardly rectifying potassium channel subunit (Kir2.1) selectively in the VTA of *Clock* Δ 19 mice using viral mediated gene transfer. Introduction of this channel reduces the firing rate of dopamine neurons in *Clock* Δ 19 mice and leads to a change in dopamine cell volume. Reduction of dopaminergic firing rates in *Clock* Δ 19 animals leads to a normalization of locomotor, and anxiety-related behavior that is very similar to lithium treatment, however it is not sufficient to reverse depression-related behavior. Similarly, restoration of a functional CLOCK protein into the VTA of *Clock* Δ 19 rescues the anxiety, but not depression-like behavioral abnormalities of these mutants.

Discussion: These results suggest a clear role for CLOCK in the VTA in the modulation of dopaminergic excitability and the regulation of anxiety related behavior. Furthermore, changes in dopamine cell morphology may ultimately underlie alterations in dopaminergic activity and anxiety-related behavior, as well as the reversal of these phenotypes by lithium treatment.

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19. Alterations of AMPA receptor expression in mice with reduced expression of SynGap

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Background: Abnormalities of the NMDA receptor signaling complex have been found in several neuropsychiatric illnesses including schizophrenia. This signaling complex is comprised of NMDA receptor subunits, structural elements such as post-synaptic density 95 (PSD95), as well as several kinases and other signaling molecules including the RasGAP signaling molecule, Synaptic GTPase-activating protein (SynGap). SynGap binds the c-terminus of PSD95, is heavily enriched in the PSD, and forms part of a biological complex that modulates GTPases and kinases. Full SynGap knock-out mice are developmentally abnormal and do not survive more than a few days after birth. Heterozygous SynGap mice have a milder phenotype with deficits in cognition, memory and social behaviors. Thus, the adult SynGap heterozygous mice are modeling the consequences of deficient NMDA receptor signaling during development. We used these animals to test the hypothesis that abnormal NMDA receptor signaling leads to other deficits in glutamate synapses, including alterations in glutamate transport, as well as changes in AMPA receptor expression and trafficking.

Methods: We used QPCR to measure transcript expression of the excitatory amino acid transporters (EAAT) 1-3, the AMPA receptor

subunits GluR1-4, and several AMPA receptor trafficking proteins, including transmembrane AMPAR regulatory proteins 2-8 (TARPs) and cornichons 1-4, in several regions of brains from wild type and SynGap heterozygous mice.

Results: We found no changes in transcripts for the glial glutamate transporters EAAT1-2 in the anterior cortex, hippocampus, striatum, or thalamus. We found decreased expression of the neuronal transporter EAAT3 in the anterior cortex but not in the other regions. We also performed glutamate reuptake assays on synaptosomes from the whole brain or from the hippocampus and found no changes in [3H]-glutamate reuptake in wild type versus heterozygous mice. In the anterior cortex, but not the striatum or hippocampus, we found decreased expression of transcripts for GluR2, GluR3, GluR4, TARP2 (aka stargazin), and TARP3. In addition, TARP5 transcripts were decreased in the thalamus.

Discussion: These data indicate that SynGap heterozygous mice have alterations in AMPA receptor subunit expression and trafficking. We postulate that developmental alterations in the NMDA receptor signaling complex leads to remodeling of the neuronal elements of synapses, including AMPARs and the neuronal transporter EAAT3. In contrast, our data suggest that astrocytes in glutamate synapses do not have alterations in glutamate transporter activity. Since the glutamate reuptake machinery is relatively intact, it may be more responsive to pharmacological manipulation as a strategy for modulating synaptic glutamate levels. We also postulate that alterations in AMPA receptor expression and trafficking in the anterior cortex might underlie the previously reported alterations in cognition, memory, and social behaviors.

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20. CRF R-1 in the Rat VTA and DRN: Critical Sites for Escalation of Cocaine and Alcohol Intake after Intermittent Exposure

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Background: Intermittent exposure to salient events such as drugs or social stress induces long-term neuroadaptive changes that escalate the self-administration of alcohol and cocaine. Preclinical evidence points to the induction of behavioral and neural sensitization as contributing to the escalation of drug taking. Corticotrophin releasing factor (CRF) is the major neuropeptide that modulates the hypothalamic pituitary axis (HPA) in response to stress and engenders mesocorticolimbic activation, which can cause modulation of dopamine and serotonin by CRF releasing neurons. The current work builds on the substantial evidence that implicates CRF R-1 subtype in the modulation of alcohol and cocaine intake. We aimed to learn whether or not CRF R-1 antagonist (CP-154,526) microinjected into the ventral tegmental area (VTA) or dorsal raphe nuclei (DRN) can selectively reduce (1) escalated alcohol drinking due to intermittent access, and (2) escalated cocaine intake due to intermittent social stress. Previously, pretreatment of a CRF R-1 antagonist (CP154,526 (20 mg/kg i.p.)) prior to social defeat stress protected against the development of social stress-induced sensitization and reduced cocaine self-administration during a 24 h "binge."

Methods: Male Long-Evans rats were submitted to four intermittent episodes of social defeat, each episode separated by two days. In each episode the intruder rat was placed into the cage of the aggressive resident rat. A group of rats were kept in their homecages and did not undergo social stress (control). Twenty minutes before social defeat stress rats received microinjections of artificial cerebral spinal fluid (aCSF) or 3 μ g CP154,526 into the VTA. Ten days after the 4th defeat, rats were challenged with cocaine (10 mg/kg, i.p.) and walking behavior was recorded to assess the expression of behavioral cross-sensitization to cocaine. The following day animals were allowed to self-administer cocaine an indwelling catheter under fixed (.75 mg/kg/infusion) and

progressive ratio (.3 mg/kg/infusion) schedules, and a 24-h continuous access “binge” (.3 mg/kg/infusion). In a separate experiment, male Long-Evans, fitted with intra-VTA or intra-DRN cannulae, rats were given access to 20% w/v alcohol and water every 48 to 72 h.

Results: Intermittent social stress escalated cocaine self-administration, particularly in the 24-h “binge.” Intermittent access to alcohol escalated alcohol intake to more than 6–7 g/kg/day in preference to water. CP154,526 microinjected into the VTA prior to each episode of social stress protected against behavioral sensitization and against stress-induced escalated cocaine taking. Similarly, intra-VTA microinjections prevented escalated alcohol intake due to intermittent access, at least for several hours, but did not affect water intake.

Discussion: These results suggest that CRF R-1 in the rat VTA is a critical site for escalated cocaine and alcohol intake, and pretreatment with CP154-526 protects preferentially against the reinforcing effects of cocaine and alcohol.

Disclosure: K. Miczek: None. C. Boyson: None. L. Hwa: None. J. DeBold: None.

21. An Animal Model of Vulnerability to Comorbid Post-Traumatic Stress Disorder and Addiction

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Background: Many psychiatric disorders are precipitated by environmental triggers, including post-traumatic stress disorder (PTSD) and drug addiction. However, only a minority of individuals who experience trauma or try addictive substances go on to develop PTSD or addiction. This implies variation in the inherent vulnerability of individuals to developing PTSD or addiction in response to a given trigger. Despite significant nosological differences, there is evidence to suggest shared vulnerability to both PTSD and addiction; the two disorders are highly comorbid and share some common neurobiological features, e.g. reduced hippocampal volume and hypoactivity of the prefrontal cortex. We investigated whether individual differences in the tendency to approach and contact a stimulus paired with food reward, thought to indicate the propensity to attribute incentive salience to reward cues, would predict exaggerated conditioned responses to a stimulus paired with foot shock in rats, a possible marker for vulnerability to PTSD.

Methods: We first used a Pavlovian conditioned approach, a.k.a. “autoshaping,” procedure to classify animals based on whether they learned to approach and interact with a retractable lever that predicted food reward (sign-trackers; STs) or learned upon lever presentation to go to the location of impending food delivery (goal-trackers; GTs). In previous studies we have shown that for STs the cue itself is attributed with incentive salience, whereas for GTs it is not. We then conducted a fear conditioning procedure in which the same rats were presented with 5 auditory tones paired with foot shock, and freezing responses were recorded by pressure-sensitive floor sensors. Rats were placed back into the same context in the absence of tones or shocks on the following day to measure freezing behavior to the conditioning context. The rats were then trained to nose-poke for a new food reward until they achieved steady response rates of ~1 Hz throughout the session. In what is known as a “conditioned suppression” test, the tone they had previously heard during fear conditioning was then played while they were responding for food, and the degree to which the tone suppressed their nose-poking response was recorded.

Results: In this experiment, 11 rats were classified as STs, 14 as intermediate responders (IRs), and 11 as GTs. STs and GTs did not differ on acquisition of conditioned freezing during tone-shock pairings ($F_{1, 32} = 0.18$, $p = 0.676$). When contextual fear was measured, GTs spent a significantly higher % time freezing than STs ($F_{1, 21} = 6.22$, $p = 0.021$). For the conditioned suppression test, there were no significant differences between groups in the number of active nose-pokes before or during the tone on the first test day, but active

nose-pokes were reduced almost to zero for all animals during the tone (ST tone = 1.1 ± 0.7 , GT tone = 2 ± 1), suggesting a possible floor effect. On the second test day, active nose-pokes were still significantly reduced for both STs and GTs during the tone (ST pre-tone = 30 ± 3 , ST tone = 8 ± 2 , $p < 0.001$; GT pre-tone = 33 ± 4 , GT tone = 17 ± 3 , $p = 0.001$), but STs had significantly fewer nose-pokes during the tone relative to GTs ($p = 0.025$), even though there were no significant differences between groups in nose-pokes before the tone ($p = 0.662$). There was a significant correlation between the conditioned approach index score used to classify STs and GTs, and the change in number of nose-pokes during the tone on the second test day ($r^2 = 0.116$, $p = 0.042$).

Discussion: In previous experiments, we have shown that STs do not differ from GTs in initial learning of a conditioned fear response, but that STs do freeze more to the tone when tested in a different context under extinction conditions. In the present experiment, as before, STs and GTs did not differ on acquisition of conditioned freezing during tone-shock pairings. However, the tone suppressed nose-poking to a greater extent in STs than GTs in a new context, again indicating greater cue-induced fear. We also found that GTs exhibited greater contextual fear than STs when placed back into the original fear-conditioning context in the absence of any temporally discrete cues. These experimental results converge to suggest that some rats attribute more motivational salience to cues predictive of food reward and to cues predictive of foot shock, while other rats make more use of context to modify their reactions to such salient stimuli. Thus, there may be a subset of individuals who tend to attribute high levels of motivational salience to predictive cues regardless of emotional valence, which may predispose them to both PTSD and addiction.

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22. Extinction of Conditioned Opiate Withdrawal in Rats in a 2-Chambered Place Conditioning Apparatus

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Background: Recently we reported that conditioned opiate withdrawal (defined operationally as avoidance of an environment previously paired with naloxone-precipitated withdrawal in morphine-dependent rats) extinguishes over the course of repeated exposures to that environment in the absence of precipitated withdrawal (Myers and Carlezon 2010). Furthermore, we found that this type of extinction is facilitated by the NMDA receptor partial agonist D-cycloserine (DCS), similar to extinction of cocaine conditioned place preference (Botreau et al. 2006) and conditioned fear (Walker et al. 2002). These findings are important because they suggest that exposure therapy for addiction might also be enhanced by DCS, a possibility that has recently received some empirical support (Santa Ana et al. 2009). In much of our work we have used a 3-chambered place conditioning apparatus comprising two equally-sized conditioning chambers separated by a smaller “start box” into which a rat is placed at the beginning of a test session. A problem inherent in the use of an apparatus of this design is that some rats tend to spend a great deal of time (as much as 50% of a test session) in the start box after conditioning. This “start box effect” is problematic because it artificially decreases the differential in time spent between the withdrawal-paired and neutral environments, since so much of the session time is spent outside of either chamber. To circumvent this problem, we have begun using a new place conditioning apparatus modeled after one used extensively by Cunningham and colleagues (1993). These boxes differ from our 3-chambered boxes in that they are 2-chambered boxes that lack a start box, and the two chambers are distinguished from one another solely on the basis of floor texture. In the present experiments we developed a protocol to study extinction of conditioned opiate withdrawal in rats using this apparatus. We have found that the parameters that worked well in the 3-chambered boxes

are not ideal in the 2-chambered boxes, and that a modified experimental protocol is needed.

Methods: The 2-chambered place conditioning apparatus consists of a black rectangular Plexiglas box positioned atop two distinctively-textured floors (constructed of perforated stainless steel and equally-spaced stainless steel rods, respectively). A removable black Plexiglas partition can be used to divide the box into two halves, each with its own floor texture. A sheet of clear Plexiglas serves as a lid. The apparatus is assembled on a cart and positioned below a camera affixed to the ceiling of the room. The data are scored by the Ethovision software system. Male Sprague-Dawley rats were implanted SC with two 75-mg morphine pellets that slow-release morphine for a period of 14 d (Gold et al. 1994). Three days later they were trained to acquire an aversion via confinement on one of the floors for 1 hr following a SC injection of naloxone (15 µg/kg). Subsequently we evaluated several different extinction training protocols, including a repeated confinement and test design similar to one we used previously (Myers and Carlezon, 2010) and a between-groups design in which rats are tested a single time following the completion of all phases of training. In both protocols, extinction training involved 30-min confinements (separated by 2-3 hrs) on each floor immediately following a SC injection of saline. Rats were tested by placing them into the center of the apparatus (with the partition removed) and allowing them to explore freely for 30 min.

Results: Overall the rats exhibited no preference for either floor prior to training, indicating that the 2-chambered apparatus is unbiased. After training, a large majority of rats avoided the naloxone-paired floor. With the design involving repeated confinements and repeated tests, extinction was exceedingly variable and subject to unconditioned effects that appeared over the course of multiple test sessions. With a between-subjects design involving a single test, extinction was orderly and reproducible.

Discussion: Problems associated with the use of a 3-chambered apparatus can be avoided by using a 2-chambered apparatus, although each apparatus requires different parameters to study extinction of conditioned withdrawal. Unconditioned effects associated with repeated testing are eliminated by using a single-test, between-groups design.

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23. Postnatal GAD67 Ablation in a Subset of Corticolimbic Interneurons Results in Behavioral Phenotypes Characteristic of Major Neuropsychiatric Disorders

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Background: Postmortem studies have consistently demonstrated reductions in the expression of GAD1 (which encodes GAD67, the enzyme responsible for activity-dependent production of GABA) in the corticolimbic areas of patients with a number of major neuropsychiatric disorders including schizophrenia, bipolar disorder and major depression. These findings have led to the hypothesis that alterations in GABAergic interneurons are involved in the etiology of neuropsychiatric disorders. Alternatively, it is plausible that GABAergic dysregulation *per se* may not determine the disease specificity. To date, it is unclear whether reduced CSF GABA, brain GAD67 levels or other markers of GABAergic dysregulation are directly related to the disease state or whether they are heritable marker for risk-factor traits. This is partly due to the difficulty inherent in creating cell-type specific targeted knockdowns of genes critical for GABAergic-interneuron functioning. Recently we have generated a line of Cre transgenic mice, namely Ppp1r2-Cre, in which Cre recombinase was selectively expressed in 40-50% of interneurons in the cortex and hippocampus (Belforte JE et al, Nat Neurosci, 2010). In the present study, we generated a conditional knockout mouse line in which Gad1 was ablated only in approximately half of cortical and

hippocampal interneurons (largely parvalbumin-type) in early post-natal development.

Methods: The ppp1r2-Cre/Gad1 homozygous KO mutant mice were directly compared to their heterozygous Gad1 mutant mice and to the Ppp1r2-Cre/NR1 mutant mice to determine the direct role of GAD67 in the development of psychiatric phenotypes. All the mice were isolated starting 1 week prior to behavioral testing and remain so throughout the behavioral assessment. These mice were subjected to a battery of behavioral tasks that each impinge on a different modality.

Results: Both heterozygous and homozygous mutants were viable and showed no gross abnormalities. Following social isolation stress, no behavioral abnormalities were detected in heterozygous Gad1 KO mutants. In contrast, homozygous Gad1 mutant mice showed many phenotypes which may be reminiscent of major neuropsychiatric disorders. These include abnormally high locomotion in the open field, pronounced hypo-activity in the home cage running wheel test, mild mating deficits, mild anxiety, impairments in social and nesting behavior, an elevated fear response and marked weight gain. Consistent with the running wheel hypo-activity the mice appeared to demonstrate a lack of intrinsic motivation. Conversely, the mice showed no robust impairment in prepulse inhibition (PPI) and no deficits in spatial working memory in the Y-maze spontaneous alternation. More precise description of behavioral phenotypes is underway.

Discussion: Despite the widely held theory that a reduction in Gad1 is primary in the etiology of schizophrenia, these results suggest that a robust reduction in cortical Gad1 expression lead not to a schizophrenia-like phenotype rather to a behavioral phenotype perhaps more consistent with mood disorders, specifically major depressive disorders. However, since a reduction in Gad67 levels are reported in many neuropsychiatric disorders and not just mood disorders, a more parsimonious explanation of the present results may be that a reduction in Gad67 leads to a phenotype consistent with an elevated neurotic personality type characterized by poor responses to environmental stress an increased propensity to interpret ordinary situations as threatening, and an increased susceptibility towards depression. This high neurotic personality in turn is known to be a risk factor for a number of neuropsychiatric disorders.

Disclosure: K. Nakazawa: None. S. Kolata: None.

24. Model of Small Cerebral Infarcts Induces Anhedonia and Alters Fractional Anisotropy

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Background: Small cerebral infarcts (SCIs) are diffuse brain lesions resulting from vascular occlusions. SCIs are estimated to affect 30% of the population over age 65 and correlate with altered mood states. Magnetic resonance imaging studies show that an estimated 60% of individuals with pre-senile onset depression and 94% of people with senile onset depression exhibit these lesions. Furthermore, SCI-induced "vascular depression" presents symptoms distinct from clinical depression and is highly resistant to antidepressant treatments. Although links between ischemia and disrupted mood have long been suggested, neither a causal relationship nor an underlying mechanism has been established. The current study uses a rat model of experimentally induced diffuse ischemic lesions to determine if SCIs are sufficient to produce depressive-like behavior. Because the elderly population is more susceptible to the behavioral and structural changes associated with SCI, this study examines outcome in both adult and aged animals.

Methods: Adult (3 mos) and aged (16 mos) male Wistar rats underwent either SHAM or SCI procedures to the left hemisphere. Following a two-week recovery, behavioral tests were conducted to assess both depressive and anxiety-like behaviors. Animals were then perfused and

brains were agarose mounted in clear tubes. Each tube was scanned in a 9.4 T Bruker Animal Scanner with a 20 cm bore. Tubes were placed in a bird cage volume coil with a 72 mm diameter. The brains underwent one T2 weighted anatomical scan (12 averages with TE = 25 ms, TR = 15 s; resolution = 0.16 x 0.16 x 0.16 mm) and two diffusion tensor imaging (DTI) scans (64 directions, resolution 0.230 mm isotropic) in a 9.4 T Bruker Dedicated Small animal scanner. DTI was used to correlate structural integrity and connectivity of brain regions implicated in SCI damage to changes in affective behavior.

Results: SCIs were more behaviorally detrimental in aged as compared to young adult rats with anhedonia as the most pronounced behavioral change. Fractional anisotropy was altered following SCI in an age and brain region specific manner. Examination of high resolution T2 scans demonstrated similar lesion volumes and locations to previous studies which used stereological endpoints.

Discussion: Correlations between SCIs and late life depression are prevalent in the literature. The results of this study complement the available clinical data by demonstrating that experimentally-induced SCIs are sufficient to cause increases in depressive-like behavior in a rat model. Furthermore, the results suggest that changes in connectivity may contribute to the behavioral effects of SCIs. Given the growing patient population suffering from late life depression, there is a critical need for a better understanding of the underlying neurobiology of the disorder which may provide novel therapeutic options.

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25. A Proteomic Discovery of a Novel Cellular Target of Lithium - Eukaryotic Elongation Factor-2 (eEF-2)

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Background: Inhibition of glycogen synthase kinase-3 (GSK-3) is thought to be a major consequence of the biological and clinical activity of the mood stabilizer lithium. However, lithium and GSK-3 may affect different cellular pathways. We employed a proteomic method to uncover new downstream targets of lithium, and then examined whether these proteins are related to GSK-3.

Methods: The proteomic study was carried out in human neuroblastoma SH-SY5Y cells treated with 20 mM LiCl for 16 h. Two-dimensional (2D) gel separation was followed by mass spectrometry analysis performed at the Smoler Proteomics Center of the Technion in Haifa, Israel. Protein levels of specific proteins were evaluated by Western blot analysis. For overexpression cells were incubated with recombinant adenovirus coding for GSK-3 α or GSK-3 β using an adenoviral vector system.

Results: Proteomic analysis identified eukaryotic elongation factor-2 (eEF-2) as a cellular target of lithium. This was verified in SH-SY5Y cells and animal models. In cells, lithium decreased eEF-2 phosphorylation at its key inhibitory site, threonine 56, and blocked the enhancement of eEF-2 phosphorylation normally coupled with stress conditions such as nutrient and serum deprivation. Unexpectedly, inhibition of GSK-3 enhanced eEF-2 phosphorylation, and overexpression of GSK-3 α or GSK-3 β resulted in a strong reduction in eEF-2 phosphorylation. Chronic administration of lithium reduced the hippocampal fraction of phospho-eEF-2 (phospho-eEF-2/total eEF-2) by twofold in two different mouse strains.

Discussion: Unexpectedly, eEF-2 is a cellular target similarly affected by both lithium and GSK-3. Lithium's reduction of eEF-2 phosphorylation does not appear to be mediated through its ability to inhibit GSK-3. Lithium's inhibition of GSK-3 is not sufficient to counteract GSK-3's effect on eEF-2 phosphorylation.

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26. Orientation and Cellular Distribution of Membrane-Bound Catechol-O-Methyltransferase in Cortical Neurons and its Implications for Drug Development and Personalized Medicine

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Background: Catechol-O-methyltransferase (COMT) is a key enzyme for inactivation and metabolism of dopamine, norepinephrine, epinephrine, caffeine, estrogen and other catechol compounds. It plays important roles in cognition, arousal, pain sensitivity and stress reactivity in human and animal models. Human genetic studies have demonstrated that the COMT gene is inconsistently associated with schizophrenia and other psychiatric disorders. A well characterized functional polymorphism, the Val158Met polymorphism, within the COMT gene affects COMT enzyme activity. The Met allele, which is a human-specific mutation, is associated with lower enzyme activity and better cognitive function but higher pain sensitivity. There are two major forms of COMT proteins, the membrane-bound (MB) COMT and soluble (S) COMT. The MB-COMT is the main form of COMT in the brain and the S-COMT is the main form of COMT in the liver. However, the cellular distribution of the MB-COMT in neurons remains unknown and the orientation of the MB-COMT on the cellular membrane of neurons is controversial. Currently available COMT inhibitors for medication inhibit both MB-COMT and S-COMT. Only one COMT inhibitor, tolcapone, shows good penetration of the blood-brain barrier, but its toxicity to liver for some patients limits its clinical applications.

Methods: *Construction of COMT-GFP fusion gene:* MB-COMT cDNA was cloned into the pEGFP-C1 and -N1 vectors (Clontech) to generate N- and C-terminal EGFP tagged COMT fusion genes, respectively. *COMT enzyme activity assay:* The assay uses the organic solvent extraction method that separates the radioactive product, the methylated catechol, and the free radioactive coenzyme, ³H-SAM (Zurcher and Da Prada 1982). *Immunocytochemistry:* Specific antibodies against secretogranin II, synaptotagmin, TGN 38, MAP2 and tau were used to stain specific markers in primary rat cortical neurons. cholera-toxin B was used to stain lipid rafts in the cortical neurons.

Results: In this study, we demonstrated that the MB-COMT is located in the cell body, the axon and the dendrites of rat cortical and hippocampal neurons. It is co-localized with Secretogranin II, a lipid raft marker, and Synaptotagmin, TGN 38, MAP2 and Tau. Our results revealed that the catalytic domain of MB-COMT is on the outer space of the cortical neurons, which suggests that MB-COMT can inactivate synaptic and extrasynaptic dopamine on the surface of postsynaptic neurons. Our drug toxicity analysis reveals that transfected HEK cells or lymphocyte cell lines with higher level of COMT enzyme activity are more resistant to the cellular toxicity of tolcapone.

Discussion: The orientation of the catalytic domain of the MB-COMT makes it possible to design MB-COMT-specific inhibitors, which do not bind to S-COMT and could be less toxic to liver. Our toxicity analysis result suggests that patients with COMT-Met genotype might be more susceptible to tolcapone toxicity and individualized medicine is important for tolcapone medication.

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27. Four Novel Factors which Induce Differentiation of Mesencephalic Dopaminergic Neurons from Human Embryonic Stem Cells

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Background: Many of the factors which induce dopaminergic (DA) neuronal specification, such as SHH, Wnt1, and FGF8, are known. In addition, various proteins, such as GDNF, BDNF, MANF, CDFN, and

PTN, are known to promote the functioning and differentiation of DA neurons. While there are several methods for inducing DA differentiation of human embryonic stem cells (ESC) involving use of these factors, it is also possible to employ stromal feeder cell lines, particularly the mouse PA6 stromal cell line, to induce the differentiation of ESC into dopaminergic (DA) neurons in a single step. The chemical nature of the factors responsible for this effect, known as SDIA, is unknown.

Methods: To identify SDIA, we carried out gene expression profiling of PA6 cells in comparison to other cell lines which lack SDIA. Factors highly expressed by PA6 cells included stromal cell-derived factor 1 (SDF-1/CXCL12), pleiotrophin (PTN), insulin-like growth factor 2 (IGF2), and ephrin B1 (EFNB1). The combination of these four factors was termed SPIE. *In vitro* functional analysis of candidate molecules on DA differentiation of hESC lines was carried out following a brief (2-4 day) embryoid body (EB) formation phase.

Results: After 10 days, the majority of colonies differentiated from BGo1V2 and BGo3-derived EBs by exposure to SPIE contained a high percentages of cells expressing the midbrain neural progenitor cell marker *Msx1* and tyrosine hydroxylase (TH). To further characterize the role of each candidate molecule in TH+ cell induction, we individually excluded each factor from the culture medium. The absence of any one factor resulted in a decrease in DA differentiation as compared to complete SPIE. Omission of SDF-1 or EFNB1 decreased the number of TH+ colonies by more than 50% in comparison to cultures exposed to SPIE. Cultures lacking PTN or IGF2 contained approximately 33% and 41% fewer TH-expressing colonies respectively. In addition, numbers of TH+ cells within colonies were greatly decreased in conditions lacking each one of the factors. RT-PCR and western blot analysis confirmed the expression of midbrain specific markers, including engrailed 1, *Nurr1*, *Pitx3*, and dopamine transporter in cultures influenced by SPIE. SPIE-induced dopaminergic neurons generated action potentials and formed functional synaptic connections. Not all hESC lines responded equally to SPIE; BGo1V2 and BGo3 formed large numbers of DA neurons, while BGo2, ESo2, and ESo4 were much less responsive. SPIE did not induce DA differentiation of various mouse neural progenitor cell lines.

Discussion: Therefore the application of the four factors SDF-1, PTN, IGF2, and EFNB1, which we have termed SPIE, to certain hESC lines results in rapid generation of DA neurons under chemically-defined conditions, free of xenogenic cells or material. PTN is a known DA neurotrophic factor; however, the mechanism through which IGF2, EFNB1 and SDF-1 induce DA differentiation is unknown. (Some of these data have previously been published; T. Vazin et al., PLoS ONE, 4(8):e6606, 2009). Research was supported by the IRPs of NIDA and NIA, NIH, DHHS.

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28. Growth Arrest and DNA-Damage-Inducible, Beta (GADD45b) Expression and Function in the Brain: An Examination of a Putative Member of a DNA Demethylation Pathway Abnormally Expressed in Psychosis

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Background: Aberrant DNA methylation within candidate gene promoter regions has been suggested to be a contributory factor to schizophrenia pathophysiology. Abnormalities associated with the addition of methyl groups have been well-characterized. However, to date no systematic investigation of proteins responsible for the removal of this reversible mark has been undertaken in psychiatry. The mechanism of active DNA demethylation in post-mitotic cells remains to be clarified. In neurons, electroconvulsive seizures (ECS), fear conditioning, depolarization, and histone deacetylase inhibitors

(HDACi) induce DNA demethylation. Previous studies have demonstrated that GADD45b is a major contributor to DNA demethylation in the brain, and its actions are rapid enough to produce an accurate signal, commensurate with brain activity. The GADD45 family of proteins may facilitate DNA demethylation as part of a two-step process involving 5-methylcytosine deamination followed by T:G mismatch repair involving DNA glycosylases.

Methods: mRNA expression of components of this demethylation pathway (i.e., GADD45 (GADD45a, GADD45b, GADD45g) and DNA glycosylase (TDG, MBD4) genes) were measured in human parietal postmortem cortical samples from the Stanley Medical Research Institute using qRT-PCR with B-Actin and TFRC as controls. Immunoblotting of GADD45b was conducted on these same samples with B-Actin as control. Mouse E14 primary cortical neuronal cultures were treated with a sodium channel agonist, NMDA, KCl, and several HDACi and a sodium channel antagonist, MK-801, and nifedipine. Changes in mRNA expression were measured using qRT-PCR with GAPDH as control and protein via immunoblotting with B-Actin as control.

Results: Our most significant finding is the increased expression of GADD45b in psychotic subjects (mean = 15.7, SD = 17.3 vs. mean = 33.6, SD = 37.9; $p = 0.04$). Increased GADD45b was confirmed using immunoblotting (mean = 4.3, SD = 2.9 vs. mean = 6.6, SD = 3.7; $p < 0.05$). In mouse primary neuronal cultures, we have found agonists of sodium channels, calcium channels, NMDA receptors, and HDACi to be capable of rapidly and robustly increasing its expression.

Discussion: The abnormal expression of a putative DNA demethylation protein, with the characteristics of an immediate early gene, in psychotic subjects is significant and warrants further investigation. However, mechanistic studies are not possible using postmortem brain samples. Therefore, we have used a mouse primary neuronal culture to characterize the stimuli that activate GADD45b expression and its necessity in coordinating demethylation.

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29. Resting Glutamate Levels and Rapid Bursts of Glutamate Release in the Prefrontal Cortex of the Flinders Sensitive Line Rat - A Genetic Rodent Model of Depression

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Background: Although the involvement of biogenic amines in major depressive disorder has been widely studied, the emerging role of glutamate is still being considered. Despite the numerous drugs targeting biogenic amines for depression the search for novel therapeutics continues due to their poor response and remission rates and slow onset of action (often 2-4 weeks). This latent onset of therapeutic efficacy suggests possible alterations in downstream targets such as the glutamatergic system. Indeed, several clinical studies have shown that the glutamatergic drugs such as the NMDA receptor antagonist ketamine and the glutamate-modulating agent riluzole are effective in treatment of recurrent major depression.

Methods: To better understand glutamate's role in depression, we utilized an enzyme-based microelectrode array (MEA) that was selective for glutamate measures with fast temporal (2 Hz) and high spatial (15 x 333 μ m) resolution. These MEAs were chronically implanted into the prefrontal cortex of 3-6 month and 12-15 month Flinders Sensitive Line (FSL) and control Flinders Resistant Line (FRL) rats, a validated genetic rodent model of depression. *In vivo* recordings of glutamate in awake, freely-moving rats were made for one hour once a stable baseline was obtained. Two-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test was used to analyze resting glutamate and spontaneous bursts of endogenous glutamate release including inter-burst interval, burst duration and burst maximum amplitude over days.

Results: Resting glutamate levels on days 3-5 post-implantation were measured for the 3-6 month FRL ($6.3 \pm 1.2 \mu\text{M}$, $5.3 \pm 1.0 \mu\text{M}$, $5.4 \pm 0.6 \mu\text{M}$; $n=7$) and FSL ($5.3 \pm 1.0 \mu\text{M}$, $5.5 \pm 0.5 \mu\text{M}$, $5.0 \pm 0.6 \mu\text{M}$; $n=6$) and the 12-15 month FRL ($3.8 \pm 1.4 \mu\text{M}$, $4.4 \pm 1.8 \mu\text{M}$, $5.2 \pm 2.7 \mu\text{M}$; $n=4$) and FSL ($19.3 \pm 3.4 \mu\text{M}$, $17.3 \pm 2.9 \mu\text{M}$, $17.4 \pm 3.7 \mu\text{M}$; $n=4$) rats. A significant increase in resting glutamate levels was observed in the 12-15 month FSL rats compared to the 3-6 month FSL ($p < 0.001$) and age-matched FRL ($p < 0.05$) rats. Our MEA also recorded a unique phenomenon in all four rat groups of transient fluctuations in glutamatergic release or "spontaneous bursts". While these events lasted only seconds, they did occur throughout the testing paradigm. The average concentration of these glutamate burst events was significantly increased ($p < 0.001$) in the 12-15 month FSL rats ($1.9 \pm 0.5 \mu\text{M}$) compared to 3-6 month FSL ($0.4 \pm 0.1 \mu\text{M}$) and age-matched FRL ($0.4 \pm 0.1 \mu\text{M}$) rats.

Discussion: Taken together, these data support that changes in the glutamatergic system underlie the pathologies observed in the FSL rat model of depression. This model provides researchers with a rodent model of depression for screening potential therapeutic treatments aimed at the glutamatergic system.

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30. Evidence for Abnormal Forward Trafficking of AMPA Receptors in Frontal Cortex of Elderly Patients with Schizophrenia

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Background: Several lines of evidence point to alterations of alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor trafficking in schizophrenia. Multiple proteins, including synapse-associated protein 97 (SAP97), glutamate receptor-interacting protein 1 (GRIP1), and N-ethylmaleimide sensitive factor (NSF), facilitate the forward trafficking of AMPA receptors from the endoplasmic reticulum toward the synapse. Once localized to the synapse, AMPA receptors are trafficked in a complex endosomal system. In this system, AMPA receptors are endocytosed in early endosomes, containing EEA1, and may be sorted to recycling endosomes, containing Rab11, or late endosomes, containing Rab7, for degradation. We hypothesize that alterations in forward trafficking and endosomal sorting of AMPA receptors may be associated with the pathophysiology of schizophrenia.

Methods: Dorsolateral prefrontal cortex was used from subjects from the Mount Sinai Medical Center brain bank. For total protein expression studies, tissue was prepared for western blots. Samples were electrophoresed in gradient gels and transferred to polyvinylidene fluoride membranes. Membranes were probed for protein using commercially available antibodies, scanned and analyzed using the Licor Odyssey laser-based image detection method. Total protein expression was normalized to beta-tubulin or Valosin-containing protein (VCP) as an in-lane loading control. Early endosomes were isolated using magnetic beads pre-incubated with an EEA1 antibody specific to early endosomes. Following isolation of early endosomes, samples were prepared for western blot as described above. Electron microscopy was used to confirm the isolation and enrichment of early endosomes in our preparation. Protein expression in early endosome isolation studies was normalized to EEA1 expression as an in-lane loading control. Data were analyzed using ANOVA or ANCOVA where appropriate.

Results: We found significant increases in expression of SAP97 and GRIP1, but not NSF in schizophrenia in total homogenate. We found no change in total homogenate expression of GluR1-3. We found no change in the expression of several endosome related proteins including EEA1, Rab7, Rab11, or GRASP1. Following isolation of early endosomes, we found significantly increased expression of GluR1, but

not GluR2 or GluR3, in early endosomes in subjects with schizophrenia.

Discussion: We found increased expression of proteins associated with AMPA receptor trafficking and an increase in GluR1 expression in the early endosomes in schizophrenia. The lack of a significant change in expression of proteins associated with endosomes suggests that the endosome sorting mechanism is intact in schizophrenia. Taken together, these data suggest that forward trafficking and subcellular localization of AMPA receptors is altered in schizophrenia and may contribute to the underlying pathophysiology.

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31. Possible Implication of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in Schizophrenia: Regulation of Spine Formation and Genetic Association

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Background: PACAP is a multifunctional neuropeptide acting as both neurotransmitter and neurotrophic factor. Our previously developed mice lacking PACAP showed marked behavioral and neurophysiological changes, including novelty-induced hyperlocomotion, deficits in prepulse inhibition, impaired memory retention, depression-like behavior as well as altered responsiveness of the HPA axis to stress. Most of these abnormalities were reversed by the atypical antipsychotic drug risperidone, while the typical haloperidol ameliorated only the hyperlocomotion and the 5-HT_{2A/2C} antagonist ritanserin was effective on the other abnormalities. In addition, a case-control association analysis of schizophrenia in a Japanese population provided evidence that genetic variants of PACAP and PACAP receptor PAC₁ genes are associated with schizophrenia. These data showed that PACAP is involved in the regulation of psychomotor behaviors and suggested that alterations in PACAP signaling might contribute to the pathogenesis of psychiatric disorders.

Methods: In order to examine whether the genetic association is also present in a Western population, we used a candidate gene-based approach to examine the association between SNPs of PACAP and PAC₁ receptor genes and psychopathology in schizophrenia. In addition, we examined the role of PACAP in regulating spine morphology in primary cultured neurons and in the PACAP mutant mice.

Results: The genetic association study showed that five SNPs in the upstream region of PAC₁ receptor gene were associated with BPRS withdrawal retardation scores in Caucasian schizophrenic patients ($P = 0.001-0.000017$), among which the SNP located at the immediate upstream region showed the lowest P value. An *in vitro* immunohistochemical study revealed that PACAP increases the size and density of postsynaptic density (PSD)-95-labeled synaptic puncta which has been linked to spine morphology by interactions with 5-HT_{2A} receptors (Jones et al. 2009) and the action of atypical antipsychotic drugs and hallucinogens via regulating dendritic trafficking and function of 5-HT₂ receptors (Abbas et al. 2009). Interestingly, the density of PSD-95-labeled synaptic puncta in the dendritic spines tended to be decreased in PACAP-deficient mice compared with wild-type mice.

Discussion: The present genetic association result supports the possibility that genetic variants in the genes of PACAP and its receptors might be a risk factor for schizophrenia. However, as their contribution to the disease is probably small, the actual mechanism that PACAP signaling is implicated in the disease remains to be identified. Several lines of evidence suggest that impaired neuronal development, including dendritic spine malformation, relates to psychiatric disorders, although the underlying mechanisms remain largely unknown. We recently demonstrated that PACAP upregulates

DISC1 expression and markedly reduces the association between DISC1 and DBZ (DISC1-Binding Zinc-finger protein), implicating the modulatory mechanism of PACAP on DISC1-dependent neurite outgrowth. Our previous study also suggested that PACAP promotes the functional coupling of neuronal NOS to NMDA receptors. It is noteworthy that PACAP was shown to have an effect on synaptic PSD-95 levels. These results, taken together, suggest that PACAP may have a strong influence on signaling networks controlling neurodevelopment, impairment of which contributes to the pathogenesis of psychiatric disorders, particularly those with psychotic features which respond to 5-HT_{2A}/D₂-based atypical antipsychotic drugs.

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32. The Association of Allelic Variation with Expression in Putative Candidate Genes for Eating Disorders and OCD: A Postmortem Microarray Study of Normal Controls

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Background: The eating disorders, anorexia nervosa (AN) and bulimia nervosa (BN), and obsessive-compulsive disorder (OCD) are complex genetic disorders thought to involve multiple genes of small effect and/or environmental factors. Both AN and BN have high rates of comorbidity with obsessive-compulsive disorder (OCD) and share a number of phenotypic characteristics with OCD, particularly perfectionism and obsessiveness, even after long-term recovery. Several promising candidate genes for AN, BN, and OCD have been identified through association and neuroimaging studies; however, a recent large-scale candidate gene association study of eating disorders failed to report significant findings (Pinheiro et al., 2010). The relative success of neuroimaging studies in comparison to association studies may be due to its role as a more proximate intermediate phenotype. The most proximate intermediate phenotype is postmortem mRNA expression, which has the added advantage of elucidating the molecular mechanism of these disorders. One brain region that has been implicated in the circuitry of all three disorders is the dorsolateral prefrontal cortex. Studying the association of allelic variation with expression in normal controls has the benefit of eliminating treatment and substance abuse confounds.

Methods: First, we conducted a comprehensive literature search using PubMed, to identify all published risk-associated single nucleotide polymorphisms (SNPs) for AN, BN, and OCD with reported statistical significance. Next, we queried the NIMH Clinical Brain Disorders Branch Human Cerebrocortical Transcriptome database, which contains mRNA gene expression data using an Illumina microarray platform in postmortem samples of dorsolateral prefrontal cortex in a lifespan cohort of non-psychiatric controls [from second trimester fetal life through to age 83 (n=272)], for available data on these previously identified risk-associated SNPs. Genotype was tested as a predictor of mRNA expression first by one-way ANOVA, and then by ANCOVA with age and RNA integrity as covariates of non-interest.

Results: From 67 positive risk-associated SNPs reported for AN, BN, or OCD in the literature, 21 of these SNPs were found in our dataset. Of these 21 SNPs, while 7 SNPs had significant genotype effects by one-way ANOVA at p < 0.05, only 3 of these SNPs, rs12574821 in contactin-5 (CNTN5), rs324420 in fatty acid amide hydrolase (FAAH), and rs3741190 in synaptotagmin XII (SYT12), survived correction for multiple comparisons at p < 0.002. Using ANCOVA for these 3 SNPs, with age and RNA integrity included in the model, we found that the risk allele (AA) for rs324420 in the gene coding FAAH was significantly associated with lower mRNA expression [F(2,266) = 8.90, p = 0.0002],

and that the risk allele (GG) for rs12574821 in CNTN5 was significantly associated with higher mRNA expression [F(2,265) = 8.30, p = 0.0003]. **Discussion:** Genetic variation that increases risk for eating disorders and/or OCD is associated with mRNA expression in DLPFC for the FAAH and CNTN5 genes in normal controls. Whether or not such associations can be detected in postmortem studies of individuals with eating disorders or OCD remains to be determined. This approach suggests a possible mechanism by which genetic variation could increase risk for eating disorders and/or OCD.

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33. Intracellular Metabotropic Glutamate Receptor 5 Function in the Hippocampus

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Background: By coupling to various intracellular cascades, metabotropic glutamate receptor 5 (mGluR5) plays an important role throughout the CNS in modulating neuronal activity and synaptic transmission. Dysfunction of mGluR5 is implicated in a variety of neurological problems including anxiety, seizures, addiction, learning and memory disorders, and Fragile X Syndrome (FXS). In FXS, a single-gene disorder characterized by mental retardation and a range of autistic features, an expanded trinucleotide repeat results in a deficiency of Fragile X Mental Retardation Protein (FMRP). FMRP normally acts as a translational repressor to oppose mGluR5 signaling; thus, the lack of FMRP in FXS leads to overactivity of mGluR5. To correct the excess mGluR5 activity, mGluR5 antagonists are currently undergoing clinical trials as a treatment for FXS. Although G-protein coupled receptors like mGluR5 are traditionally thought to initiate their signaling cascades from the cell surface, there is mounting evidence that intracellular receptors, located on the nuclear membrane and endoplasmic reticulum, are also physiologically significant. Notably, up to 90% of mGluR5 is intracellularly located, where it gives rise to unique calcium responses and downstream signaling cascades in dissociated striatal cultures. The native ligand of mGluR5, glutamate, can cross the cell membrane via various transporters and exchangers, allowing glutamate released at a synapse to potentially activate both cell surface mGluR5 as well as intracellular mGluR5.

Methods: Because many studies of FMRP have been done in the hippocampus, we have extended our investigation of intracellular mGluR5 to this brain region using dissociated hippocampal cultures and acutely-prepared hippocampal slices. Immunocytochemistry, calcium imaging, and immunoblotting were performed to quantify mGluR5 expression and evaluate its function. A pharmacological approach using combinations of permeable and impermeable agonists and antagonists was used to activate cell surface mGluR5, intracellular mGluR5, or both.

Results: Immunocytochemical analysis revealed that mGluR5 is present on intracellular membranes of 45-50% of dissociated hippocampal neurons and in isolated nuclei from hippocampal tissue. Using the impermeable mGluR5 antagonist LY393053 to block cell surface activation, application of the cell-permeable agonist quisqualate led to receptor-specific Ca²⁺ changes that could be blocked by the cell-permeable antagonist MPEP, indicating that intracellular mGluR5 is functional. Similarly, activation of intracellular mGluR5 is able to increase FMRP levels in hippocampal neurites, as does cell surface mGluR5 activation, again supporting the idea that intracellular receptors are physiologically relevant.

Discussion: Defining the role of intracellular mGluR5 activation is important both conceptually, in showing the physiological relevance of intracellular G-protein coupled receptors, and clinically, in targeting drugs to intracellular receptors, cell surface receptors, or both. For Fragile X Syndrome and related neuropsychological conditions like

autism, understanding the role of intracellular mGluR5 will be beneficial in developing suitable mGluR5 antagonists for therapeutic treatments.

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34. Fgf14 Interaction With Voltage-Gated Sodium Channels Is Phosphorylation-Dependent

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Background: The brain relies on the integrity of the pore-forming α subunit of the voltage-gated Na^+ (Nav) channels as key substrate of axonal and dendritic excitability. Dysfunction of these channels is linked to a plethora of brain disorders still lacking effective therapeutic options. In pursuit of new functional proteomic and drug discovery goals, we have explored the Nav channelosome as a new source for therapeutic targets against Nav channelopathies. We have previously demonstrated that fibroblast growth factor 14 (FGF14) is a biologically relevant component of the neuronal Nav channelosome. Through an interaction with the intracellular C-terminal tail of Nav channel α subunits, FGF14 acts as a unique chaperone-like molecule controlling gating properties and subcellular localization of native Nav channels at the axonal initial segment (AIS), the site of action potential initiation. The goal of this study was to identify new cellular pathways upstream of the FGF14:Nav channel complex as an initial step toward targeted interventions aimed at restoring neuronal excitability in Nav channel-driven brain diseases.

Methods: We have applied the split luciferase complementation assay (LCA) to detect direct interaction between FGF14 and the C-terminal tail of the neuronal Nav1.6 channel real-time in living cells. In this reporter system, two complementary N-terminus (NLuc) and C-terminus (CLuc) fragments of luciferase, which have no activity on their own, were respectively fused to FGF14 (CLuc-FGF14) and to a chimera of the CD4 transmembrane segment and the C-tail of the Nav1.6 channel (CD4-Nav1.6-NLuc). CLuc-FGF14 and CD4-Nav1.6-NLuc plasmids were transiently co-expressed in HEK-293 cells and interaction was detected upon D-luciferin addition as luminescence. Cells were preincubated for 2 hours prior luminescence readings with selective kinase inhibitors and the effect of the compounds was tested as change in luminescence compared to control. Co-immunoprecipitation studies were conducted in HEK-293 cells stably expressing full length Nav1.2 channels and transiently transfected with a plasmid vector encoding Myc-tagged-FGF14. Additional studies aiming at validating the effect of selective kinase inhibitors on the FGF14:Nav channel complex were conducted quantitative immunolabeling of native FGF14 and Nav channels in primary hippocampal neurons.

Results: Expression of the CLuc-FGF14 and CD4-Nav1.6-NLuc constructs in HEK-293 cells produced a robust increase in luminescence upon addition of the substrate D-luciferin compared to the negative control, indicating association between FGF14 and Nav1.6 channel C-tail. We used this reporter system to screen an array of protein kinase inhibitors for their ability to influence the interaction between FGF14 and Nav1.6 channel. We have identified compounds which produced a significant reduction in the output signal compared to the untreated control, suggesting that inhibition of specific kinases alters the FGF14:Nav channel complex stability. Among the positive hits, we selected the casein kinase 2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) and confirmed the LCA results by co-immunoprecipitation of FGF14 and full length Nav1.2 channels in HEK293 cells. Furthermore, using quantitative immunofluorescence we demonstrated that exposure of primary hippocampal neurons to TBB (50 μM , 8 hours) causes subcellular redistribution and dispersal of FGF14 from the AIS.

Discussion: These results suggest a model in which inhibition of specific kinases might result in loss of Nav channel function and excitability through an FGF14-mediated mechanism. While extending the current knowledge of the Nav channelosome, these results reveal a potential innovative opportunity for drug discovery and therapeutic interventions targeting neuronal excitability through the FGF14:Nav channel complex within a phosphoproteomic network.

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35. Targeting Protein Kinase Inhibition to Optimize Serotonin 1B Receptor Function

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Background: Serotonin 1B receptor (5HT_{1B}R) is a Class A, G protein-coupled receptor (GPCR) that activates the inhibitory G protein (G_i) to inhibit adenylyl cyclase and reduce cAMP production. 5HT_{1B}R functions via its autoreceptor at the axon terminals of serotonin neurons to negatively regulate serotonin release. It also locates in many other types of neurons and acts as heteroreceptor to regulate neuronal activity. Although 5HT_{1B}R is thought to have important influence in serotonin-regulated brain functions, such as mood, anxiety, and reward, the characteristics of 5HT_{1B}R activation and regulation have not been fully studied, and there is no 5HT_{1B}R-targeting therapeutic agent available to date in the treatment of major psychiatric illnesses. We previous found that Glycogen synthase kinase-3 β (GSK3 β) interacts with and phosphorylates 5HT_{1B}R at the serine-154 site of its intracellular loop-2. GSK3 β is a protein kinase that phosphorylates substrate proteins to activate or inhibit their activity. Abnormal GSK3 β activity is involved in mood disorders, the therapeutic agent lithium is a selective inhibitor of GSK3, and other more potent and selective GSK3 inhibitors have anti-hyperactive and anti-depressive properties. We hypothesized that GSK3 β is a selective modulator of 5HT_{1B}R, and inhibition of GSK3 β may optimize the actions of 5HT_{1B}R agents in therapeutics of psychiatric disorders. In this study, we examined the effects of GSK3 β in 5HT_{1B}R coupling to its signaling pathways, as well as the 5HT_{1B}R-regulated serotonin release and behaviors.

Methods: In cultured cells expressing 5HT_{1B}R or 5HT_{1A}R, coupling of these receptors to G_{i2} and β -arrestin2 was examined using BRET and immunoprecipitation assays. The activities of cAMP, Akt, and Erk were measured by ELISA and immunoblotting. 5HT_{1B}R trafficking was examined by immunohistochemistry. The effect of GSK3 β on 5HT_{1B}R function was tested using mutant 5HT_{1B}R, GSK3 β -targeting shRNAs, overexpressed inactive GSK3 β , and GSK3 inhibitors. 5HT_{1B}R-regulated serotonin release was examined in mouse cerebral cortical slices preloaded with ³H-serotonin. Slices were treated with the 5HT_{1B}R agonist anpirtoline with or without a GSK3 inhibitor, followed by measuring potassium-evoked serotonin release. The behavioral effects of 5HT_{1B}R were examined by the tail suspension test and the locomotor activity in C57BL/6 mice. Mice were pre-treated with intracerebroventricular infusion of a GSK3 inhibitor followed by intraperitoneal anpirtoline treatment prior to behavior tests.

Results: In 5HT_{1B}R-expressing cells, interaction between 5HT_{1B}R and G_{i2} at the resting state was not affected by GSK3 β . Activation of 5HT_{1B}R by serotonin caused time-dependent conformational change of 5HT_{1B}R- G_{i2} interaction. This agonist-induced dynamic interaction was fully dependent on the presence of active GSK3 β since ablating GSK3 β binding to 5HT_{1B}R, knocking down GSK3 β with shRNA, overexpressing inactive GSK3 β , and treating cells with GSK3 inhibitors all silenced the agonist-induced dynamic interaction between 5HT_{1B}R and G_{i2} . In contrast, GSK3 β had no effect in serotonin-induced

dynamic interaction between 5-HT_{1A}R and G α 2. Furthermore, serotonin-induced recruitment of β -arrestin2 to 5HT_{1B}R was not affected by GSK3 β . In agreement with the above findings, GSK3 β also had differential effects in 5HT_{1B}R-regulated cAMP, Akt, and Erk activity, and 5HT_{1B}R trafficking. In mouse cerebral cortical slices, 5HT_{1B}R agonist anpirtoline reduced potassium-evoked ³H-serotonin release, and the GSK3 inhibitors, AR-A014418 and 5-ING-135, attenuated the effect of anpirtoline. In C57BL/6 mice, GSK3 inhibitors AR-A014418 and 5-ING-135 differentially modulate the effects of anpirtoline in the tail suspension test and in the rearing activity tested in the open field.

Discussion: Results of this study demonstrated: 1) GSK3 β selectively modulates serotonin-induced activation of 5HT_{1B}R, but not 5HT_{1A}R; 2) inhibition of GSK3 β does not extinguish 5HT_{1B}R activity, instead, selectively eliminates the Gi-mediated signaling pathway and reserves the β -arrestin2 action; 3) inhibition of GSK3 blocks the negative regulation of serotonin release by 5HT_{1B}R; and 4) inhibition of GSK3 diversely modulates 5HT_{1B}R-associated behaviors in mice, which may be a result of selective regulation of brain region- and neuron-specific behavioral effects of 5HT_{1B}R. Therefore, GSK3 β is a selective and active modulator of 5HT_{1B}R function, and inhibition of brain GSK3 β may optimize the effects of 5HT_{1B}R agonists and antagonists for clinical therapeutic implications.

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36. Schizophrenia, Amphetamine-Induced Sensitized State and Acute Amphetamine Exposure all Show a Common Alteration: Increased Dopamine D2 Receptor Dimerization

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Background: All antipsychotic work via dopamine D2 receptors (D2Rs), suggesting a critical role for D2Rs in psychosis; however, there is little evidence for a change in receptor number or pharmacological nature of D2Rs. Recent data suggest that D2Rs form dimers *in-vitro* and *in-vivo*, and we hypothesized that schizophrenia, as well as preclinical models of schizophrenia, would demonstrate altered dimerization of D2Rs, even though the overall number of D2Rs was unaltered.

Methods: We measured the expression of D2Rs dimers and monomers in patients with schizophrenia using Western blots, and then in striatal tissue from rats exhibiting the amphetamine-induced sensitized-state (AISS). We then examined the interaction between D2Rs and the dopamine transporter (DAT) by co-immunoprecipitation, and measured the expression of dopamine D2^{High} receptors with ligand binding assays in rat striatum slices with and without acute amphetamine pretreatment.

Results: We observed significantly enhanced expression of D2Rs dimers ($277.7 \pm 33.6\%$) and decreased expression of D2Rs monomers in post-mortem striatal tissue of schizophrenia patients. We found that amphetamine facilitated D2Rs dimerization in both the striatum of AISS rats and in rat striatal neurons. Furthermore, amphetamine-induced D2Rs dimerization may be associated with the D2R-DAT protein-protein interaction as an interfering peptide that disrupts the D2R-DAT coupling, blocked amphetamine-induced upregulation of D2Rs dimerization.

Discussion: Given the consistent increase in D2R dimers in brains from patients with schizophrenia, in animal models and in pharmacological challenges modeling; and given the efficacy of D2R blockers in schizophrenia - increased D2R dimers provide a very plausible link to the pathophysiology of schizophrenia and provide a promising new target for novel antipsychotic drugs.

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37. Novel Targets of the Transcriptional Coactivator PGC-1 α in the Brain: Implications for Neurological Disorders

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Background: The transcriptional coactivator peroxisome proliferator activated receptor γ coactivator 1 α (PPARGC1A or PGC-1 α) has been identified as a master regulator of metabolism in peripheral tissues, and it has been assumed that PGC-1 α has a similar role in the brain. We have previously shown that PGC-1 α becomes selectively localized to GABAergic interneurons by the second week of life during the development of the rodent brain. More recent evidence from our lab suggests that PGC-1 α is required for normal expression of the calcium-binding protein parvalbumin within this cell population, providing the first evidence for a non-metabolic, neuron-specific role for PGC-1 α . We hypothesized that PGC-1 α may serve to regulate other neuronal targets in a cell-specific manner in the brain and that deletion of PGC-1 α in specific cell populations in the brain may manifest in behavioral abnormalities indicative of neuronal dysfunction.

Methods: To identify novel neuronal targets of PGC-1 α , we used an unbiased oligonucleotide array approach in neuroblastoma cells overexpressing PGC-1 α . Gene and protein expression of putative array-identified targets was measured in whole-body PGC-1 α ^{-/-} mice using quantitative real-time PCR and immunohistochemistry. To determine the cellular specificity of gene regulation of PGC-1 α , gene expression was also measured in mice with neuron- and interneuron-specific deletion of PGC-1 α . Behavioral abnormalities were investigated in whole-body, neuron-, and interneuron-specific PGC-1 α ^{-/-} mice using open field, rotarod, prepulse inhibition, and Barnes maze.

Results: 1067 out of 30,000 tested transcripts (Illumina Human 6v3 array) were upregulated over two-fold, and subsequent analysis was restricted to genes with brain expression patterns similar to that of PGC-1 α (based on the Allen Brain Atlas; 19 hits). Array-identified targets included genes involved in synaptic structure and function as well as metabolism. Regional analysis of gene and protein expression in PGC-1 α ^{-/-} compared to ^{+/+} animals revealed complexin 1 and neurofilament heavy chain to be globally reduced, while synaptotagmin II and isocitrate dehydrogenase 3 α were selectively reduced in the cerebrum. Other genes were reduced to a lesser, region-specific extent, and the most gene expression changes were found in the striatum, including reductions brain protein 17 (Pnkd) and monoamine oxidase B. In whole-body and neuron-specific PGC-1 α ^{-/-} mice, motor abnormalities reminiscent of neurological disorders, such as pronounced rotarod deficits, hind limb claspings, and resting tremor, were found within the first month of life and precluded the use of these animals in traditional paradigms of learning and memory. Interneuron-specific deletion produced no overt phenotype until six months of age, at which point rotarod performance worsened. Sensorimotor gating and learning tasks are currently being investigated in these animals.

Discussion: We have identified several novel targets of PGC-1 α in the brain using both *in vitro* and *in vivo* methods. These genes are likely to be selectively regulated in neurons, as neuron-specific deletion of PGC-1 α was able to completely recapitulate the phenotype of whole-body PGC-1 α ^{-/-} mice. As PGC-1 α has been implicated in neurological disorders, such as Huntington and Parkinson Diseases, and mice lacking PGC-1 α have reductions in key synaptic, structural, and metabolic genes as well as severe motor abnormalities, PGC-1 α may hold promise as a potential therapeutic target in such illnesses.

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38. Stress-Modulated Expression of Polyspecific Cation Transporters in the Brain: Role in Individual Vulnerability to Psychopathology

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Background: The organic cation transporter-3 (OCT3) is a corticosterone-sensitive monoamine transporter that has been implicated in a variety of psychiatric disorders. The OCT3 participates in serotonin transport in the limbic system and occlusion by the stress hormone corticosterone increases extracellular serotonin, an important neuro-modulator of mood states. Altered expression of the OCT3 is associated with maladaptive behavior and may be influenced by genetic and environmental factors. In this study, we investigated the effects of stress on OCT3 expression in the brain, which in turn may regulate stress responsiveness and emotional behavior. Our goal is to ultimately identify new molecular targets that may be suitable for antidepressant therapy in individuals with poor response to currently available treatments, so we investigated the effects of the OCT3 antagonist decynium 22 on behavior in the forced swim test.

Methods: In experiment 1, we used the Wistar-Kyoto (WKY) rat as a model of depressive-like behavior and the Long-Evans (LE) rat as a comparison strain. Rats were randomly assigned to control, acute and chronic stress groups. Chronic stress consisted of a 7-day social defeat procedure followed by restraint stress on day 8. Acute stress consisted only of restraint on day 8, while control animals received no stress. Brains were collected 2 or 3 hours after the last stressor (restraint) for molecular analysis of gene and protein expression, respectively. Gene expression of the OCT3, glucocorticoid receptor (GR), and serotonin transporter (SERT) was quantified by real-time PCR and protein expression of the OCT3 and brain-derived neurotrophic factor (BDNF) was quantified by Western blot in dissected brain regions including the hippocampus, medial prefrontal cortex (mPFC) and striatum. In experiment 2, we administered decynium 22 (0, 1, 5 and 10 ug/kg, ip) to WKY rats 1h before testing on the forced swim test. Swimming, climbing, and floating behavior was recorded and the percentage of time spent immobile was used as an index of depressive behavior.

Results: We found that OCT3 gene expression in the hippocampus is upregulated by acute stress in LE rats and chronic stress in WKY rats. No effects of stress on OCT3 expression were observed in the striatum. GR gene expression, which was used as a positive control for stress-induced neuroplasticity in the hippocampus, was found to increase in WKY rats after chronic stress. No significant changes in SERT gene expression were observed in WKY or LE rats after stress. Hippocampal protein expression of the OCT3 is also increased after chronic stress in WKY rats, but was localized to the cytosol instead of the plasma membrane. WKY rats in the chronic stress group actually had reduced OCT3 protein expression at the plasma membrane. One possibility is that the binding of corticosterone to OCT3 causes it to be internalized and transported to the cytosol. We also observed an increase in BDNF expression in the hippocampus in both LE and WKY rats after chronic stress, which is consistent with the fact that stress induces neural plasticity in this brain region. We also found that the OCT3 antagonist decynium 22 has antidepressant effects in WKY rats on the forced swim test, indicating that the OCT3 may hold promise as a new molecular target for antidepressant pharmacotherapy.

Discussion: Our data indicate that OCT3 gene expression is modulated by stress-induced glucocorticoid signaling in the hippocampus, which may in turn mediate adaptive neuroendocrine and behavioral responses to stress. We have demonstrated differential regulation of OCT3 expression in WKY rats, which display depressive-like behavior and poor stress coping, indicating that the OCT3 may play a critical role in stress adaptation. Our studies also demonstrate that OCT3 antagonists are potent antidepressant agents in WKY rats and may yield similar results in humans who, like the WKY rat, are refractory to treatment with selective serotonin reuptake inhibitors (SSRIs).

Disclosure: C. Marcinkiewicz: None. D. Devine: None.

39. AMPA Receptor Associated Proteins From the TARP and Cornichon Families are Dysregulated in Frontal Cortex in Schizophrenia

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Background: The glutamate hypothesis of schizophrenia suggests abnormal glutamatergic neurotransmission in this illness. While there are multiple theoretical reasons that AMPA receptors have been implicated in schizophrenia, most studies have found normal levels of the subunits associated with the AMPA receptor in many brain regions in the illness. We have been exploring other potential sites of AMPA receptor dysregulation in postmortem brain. Previous studies in our laboratory have demonstrated abnormalities in the expression of TARP γ -2 (stargazin) mRNA in the brain in schizophrenia. TARP γ -2 is one of eight members of the transmembrane AMPA regulatory protein family. It has been proposed that the TARP γ proteins are involved in the trafficking and function of AMPARs at the excitatory synapse. Additionally, recent studies suggest AMPAR interaction with and regulation by another class of related AMPAR-auxiliary proteins, the cornichons (CNIH1-4).

Methods: We utilized quantitative real-time PCR (qPCR) to measure the expression of TARP γ -1-8 and CNIH 1-4 in two cortical areas, the anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) in postmortem brain samples from persons with schizophrenia and a comparison group.

Results: We found increased expression of TARP γ -2, γ -4, cornichon 1, cornichon 2, and cornichon 3 in the DLPFC, and decreased expression of TARP γ -4 and γ -8 in the ACC in schizophrenia. Additionally, we examined TARP γ and cornichon expression in the prefrontal cortex of rats treated chronically with haloperidol. This yielded no changes in gene expression, suggesting that TARP γ and cornichon gene dysregulation in schizophrenia is likely not due to antipsychotic treatment but rather the illness itself.

Discussion: These data add to the growing literature in support of glutamatergic abnormalities in Schizophrenia. Alterations in the expression of these genes may contribute to the pathophysiology of this illness and provide insight into potential mechanisms of glutamatergic dysregulation in schizophrenia. These results suggest that there are changes in glutamate receptors in schizophrenia that involve abnormalities of intracellular processes, such as expression of accessory proteins, that effectively change receptor function even though total cellular levels of the receptors themselves may be normal. Such findings are important because they point to the complexity of molecular and intracellular abnormalities in schizophrenia, and highlight novel sites that may be profitably targeted for drug discovery.

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40. Ethanol-Mediated Trafficking of GABA-A Alpha-1 and GABA-A Alpha-4 Receptor Surface Expression Is Reversed by Protein Kinase A Activation in Cultured Cerebral Cortical Neurons: A New Strategy to Ameliorate Ethanol Withdrawal

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Background: Ethanol exposure causes central nervous system hyper-excitability associated with internalization of GABA-A α 1 subunit-containing receptors and increased surface expression GABA-A α 4 receptors both *in vivo* and *in vitro*. These adaptations are associated with diminished synaptic inhibition that involves a reduction of the decay tau of miniature inhibitory post synaptic potentials and the loss of zolpidem enhancement of GABA responses. We recently reported that the ethanol-induced alterations in GABA-A receptor surface

expression in cultured cortical neurons are dependent upon protein kinase C (PKC) gamma, however ethanol activates PKC several hours prior to the alterations in GABA-A receptor trafficking. Thus, we postulated that other mechanisms may contribute. Since ethanol is also known to activate protein kinase A (PKA), we explored the effects of ethanol on PKA activity and the role of PKA in ethanol effects on GABA-A receptor adaptations.

Methods: Cultured cerebral cortical neurons were prepared from rat pups on postnatal day 1 as previously described (Kumar et al, 2010). Cells were maintained in culture for 15-18 days, allowing for full maturation of GABA-A receptors. Neurons were exposed to ethanol and/or PKA activators and inhibitors and surface receptors were isolated by biotinylation and/or subcellular fractionation. PKA expression was studied in P2 fractions of cell membranes.

Results: As previously reported, ethanol altered GABA-A $\alpha 1$ and $\alpha 4$ receptor expression after 4 hours, but not after one hour. Ethanol increased PKA RII α and PKA RII β expression in P2 fractions of cortical neurons at 1 hour, but these effects were absent after 4 hrs. Further, the PKA activator Sp-adenosine 3',5'-cyclic monophosphothioate triethylamine had the opposite effects of PDBu on both GABA-A $\alpha 1$ and $\alpha 4$ receptors, increasing surface expression of $\alpha 1$ subunits (~70%) and decreasing expression of $\alpha 4$ receptors (~39%). Thus, we hypothesized that opposing effects of PKA and PKC maintain GABA-A receptor expression during the first hour of ethanol exposure. Indeed, ethanol exposure for 1 hr in the presence of the PKA inhibitor Rp-adenosine 3',5'-cyclic monophosphothioate triethylamine reduced GABA-A $\alpha 1$ receptor expression by ~35% and increased GABA-A $\alpha 4$ receptors by ~44%. Conversely, ethanol exposure for 1 hr in the presence of the PKC inhibitor calphostin-C increased GABA-A $\alpha 1$ receptor expression by ~82% and reduced GABA-A $\alpha 4$ receptors (~37%).

Discussion: These results show that ethanol regulation of GABA-A $\alpha 1$ and $\alpha 4$ receptor surface expression involves both PKA and PKC activation in cultured cortical neurons. PKA activation delays the ultimate effects of ethanol on GABA-A receptor trafficking that are associated with loss of synaptic inhibition. These results suggest that PKA interactions with GABA-A receptors may prevent or ameliorate GABA-A receptor adaptations and possibly symptoms of ethanol dependence/withdrawal. Future studies will address this possibility *in vivo*.

Disclosure: A. Morrow: None. S. Kumar: None. A. Fetzer: None. D. Werner: None.

41. Assessing Interview Quality and Scoring Accuracy in Clinical Trials with Continuous Quality Control (CQC)

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Background: CNS clinical trials fail more often than their *a priori* powering indicates they should. Quality assurance/quality control (QA/QC) safeguards for clinical (including primary) outcome measures have rarely been utilized. The large number of raters performing assessments in multi-site trials increases the probability of variability in ratings. Rater drift over time is well-documented and ubiquitous, and superior interviews as measured by the Rater Applied Performance Scale (RAPS), were associated with drug-placebo separation². The RAPS is a scale used to rate interviewing skills on the domains of adherence to the interview guide, use of follow-up questions, clarification, neutrality, and research rapport. We report early findings using Continuous Quality Control (CQC), a new approach to monitoring and remediating the administration and scoring of clinical outcome measures via the RAPS.

Methods: 26 calibrated quality reviewers were rigorously trained and continuously calibrated on scale scoring and interview quality. This cohort was tightly calibrated on the MADRS, HAM-A and HAM-D, with ICCs = .91-.94 on observed interviews. The reviewers were also calibrated on assessing interview quality via the RAPS and on delivery

of feedback. Data from two on-going clinical trials were pooled. 129 Site raters audio recorded all MADRS, and HAM-A/HAM-D (SIGH-AD) administrations and uploaded the recordings to a central server. A subset of these interviews was selected for review by calibrated quality reviewers based upon a pre-determined algorithm. *A priori* scoring accuracy and RAPS interview quality criteria were established. The reviewers listened to the recorded interviews in full and independently scored 797 site raters' assessments and rated interview quality using the RAPS. Only after the reviewers' scores and RAPS ratings were locked was the reviewer given access to the site raters' scores. Detailed feedback was provided to the site raters on both interview quality (covering each domain of the RAPS) and scoring before their next reviewed assessment.

Results: 797 assessments were reviewed. At the first review of each of the 129 site raters, 56% met the *a priori* criteria for scoring accuracy, 64% for interview quality and 44% met both criteria. By review nine or later (n = 136) there were substantial improvements: 74% met criteria for scoring accuracy, 84% for interview quality and 68% met both criteria. Improvements generally occurred by month four of the study. Analysis of RAPS domains showed that inadequate interview follow-up was the most common contributor to poor interview quality. Substantial improvements were shown in Adherence, Follow-up, Clarification and Neutrality as rated on the RAPS comparing review one to review nine. Additional data are currently being collected.

Discussion: QA/QC of clinical assessments identified significant scale administration and scoring issues. Repeated feedback improved rater performance substantially over the course of the trial. This is in contrast to the well-documented phenomenon of rater drift seen in trials without QA/QC safeguards. Scoring and interview quality may require ongoing monitoring and training to achieve and maintain an acceptable standard. Study outcomes will be evaluated to determine if continuous QA/QC of study assessments assists sponsors in identifying risks that contribute to CNS trial failures.

Disclosure: B. Brown: Part 1; MedAvante. Part 2; MedAvante. Part 3; MedAvante. Part 5; MedAvante. S. De Santi: Part 1; MedAvante, Bayer. Part 2; MedAvante, Bayer. Part 3; MedAvante, Bayer. Part 5; MedAvante, Bayer. M. Detke: Part 1; MedAvante, Eli Lilly. Part 2; MedAvante, Eli Lilly. Part 3; MedAvante, Eli Lilly. Part 5; MedAvante, Eli Lilly. J. Williams: Part 1; MedAvante. Part 2; MedAvante. Part 3; MedAvante. Part 5; MedAvante.

42. Rate of Enrollment and Treatment Outcome in Schizophrenia Clinical Trials

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Background: In the past decade, results of schizophrenia clinical trials have shown an increasing placebo response rate, and smaller drug-placebo effect sizes. The trend is especially notable in the US compared to non-US regions (Chen et al, 2010). The reasons for the reduction in effect size are uncertain, though various variables have been suggested, including changes in site characteristics, changes in recruitment (types of patients and recruitment procedures), and changes in trial design (Kemp et al, 2008). There is some evidence that rate of enrollment may play a role (Liu et al, 2008), but variable has not been directly examined for clinical trials in schizophrenia. The goal of this analysis was to examine the potential influence of enrollment rate at individual study sites on placebo response, and drug-placebo difference, in two randomized, double-blind, placebo-controlled trials in schizophrenia. **Methods:** Enrollment rates and baseline-to-endpoint change in the PANSS total score (the primary outcome) were analyzed based on combined US data from two recently completed randomized, double-blind, placebo-controlled, 6-week, global phase 3 trials of lurasidone in patients with an acute exacerbation of schizophrenia: the Pearl 1 study (21 US sites; N = 278 patients; fixed daily doses of lurasidone 40 mg, 80 mg, 120 mg and placebo); Pearl 2 study (25 US sites; N = 284

patients; fixed daily doses of lurasidone 40 mg, 120 mg and placebo). The trials were conducted in 2008 and 2009, and the 46 US sites represented 45% of all global sites. For each site, the relationship between rate of enrollment per month of study participation and baseline-to-endpoint change in PANSS total score was analyzed for the placebo group. The relationship was also analyzed between enrollment rate and the difference in endpoint change in PANSS total scores for lurasidone and placebo. Since each trial included multiple fixed dose lurasidone groups, the largest improvement in PANSS total group was used to assess difference from placebo at the site. Pearson correlation coefficients were obtained between enrollment rates and PANSS change scores.

Results: Enrollment rates by site ranged from 0.6 to 7.1 patients per month, with a mean rate of 2.0 per month. Among placebo-treated patients, there was a small correlation between higher enrollment rate and lower endpoint improvement in the PANSS total score ($r = 0.221$). Among patients treated with lurasidone, there was a small correlation ($r = 0.194$) between enrollment rate and the lurasidone vs. placebo difference in endpoint change on the PANSS total score (a higher enrollment rate was non-associated with a greater reduction in PANSS total score compared to placebo). On a subanalysis that excluded the five sites that enrolled <5 randomized subjects, the correlation became even smaller between enrollment rate and placebo response on the PANSS ($r = 0.043$) and enrollment rate and lurasidone vs. placebo difference in the PANSS ($r = 0.025$).

Discussion: For US sites involved in PEARL schizophrenia clinical trials program, correlation was small between enrollment rate and the magnitude of both placebo improvement on the PANSS and lurasidone vs. placebo difference scores. Therefore in this dataset, no significant relationship was found between these two variables. Analysis of further datasets are needed to understand whether these findings are generalizable to other clinical trials in schizophrenia.

Disclosure: A. Kalali: Part 1; Cypress Bioscience, Merck, Novartis. Part 2; Cypress Bioscience. Part 5; Quintiles Inc. J. Cucchiaro: Sunovion, Inc. D. Simonelli: Sunovion, Inc. J. Hsu: Sunovion, Inc. A. Loebel: Sunovion, Inc.

43. A Double-Blind, Placebo-Controlled Trial of Topiramate for the Treatment of Comorbid Alcohol and Cocaine Dependence

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Background: The co-occurrence of alcohol and cocaine dependence is very common. Patients with both cocaine and alcohol dependence tend to have more psychosocial problems and worse treatment outcomes compared to patients addicted to cocaine or alcohol alone. The combined use of alcohol and cocaine is fostered by a variety of factors including conditioning and pharmacodynamic interactions between alcohol and cocaine. Effective pharmacological treatments for combined alcohol and cocaine dependence should include treatments effective for both addictions. Topiramate may be such a treatment. Topiramate is a novel antiepileptic drug that increases GABAergic activity in the brain, as well as antagonizes the AMPA/kainate subtype of glutamate receptors. Through these two mechanisms of action, topiramate could be useful in reducing both alcohol and cocaine use by reducing alcohol and cocaine reward as well as by reducing alcohol and cocaine craving. Topiramate has been shown to reduce alcohol use among alcoholics in several clinical trials. Topiramate has also been shown to reduce cocaine use in cocaine dependent patients in one controlled clinical trial. The current trial was intended to test the ability of topiramate to foster alcohol and cocaine abstinence among patients addicted to both alcohol and cocaine.

Methods: The study was a double-blind, placebo-controlled, trial involving 170 DSM-IV alcohol and cocaine dependent, treatment-seeking subjects. After achieving a period of abstinence from both alcohol and cocaine, subjects were randomized to topiramate, titrated

over 8 weeks to 300 mg daily, or identical placebo capsules. Medications were continued at full dose for 5 weeks then tapered during the last week of the trial. Medications were given in conjunction with twice weekly individual cognitive-behavioral relapse prevention psychotherapy, augmented with medical management to improve medication adherence. Primary outcome measures included alcohol use, measured by the timeline follow back, and cocaine use, measured by self-report and confirmed by thrice weekly urine drug screens. Secondary outcome measures included cocaine and alcohol craving, and addiction severity measured by the Addiction Severity Index.

Results: One hundred and fifty subjects completed the trial. Preliminary analyses revealed that topiramate-treated subjects, compared to placebo-treated subjects, were significantly more likely to be abstinent from alcohol (45% vs. 28%) and cocaine (17% vs. 7%) during the last three weeks of full dose medication. Complete analyses will be available at the meeting.

Discussion: Topiramate plus cognitive behavioral therapy may be efficacious for the treatment of comorbid alcohol and cocaine dependence.

Disclosure: K. Kampman: Part 4; Titan Pharma. H. Pettinati: None. K. Lynch: None. A. Jones: None. K. Varillo: None. C. O'Brien: None.

44. Clinical Trial Design Issues Affecting the Assessment of the Pro-Cognitive Effects of Broad Spectrum Antipsychotics: Examples from 12-Week Extension Data from the Phase 2b EAGLE Trial of CYP-1020

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Background: The focus of current consensus guidelines for clinical trials of neurocognitive drugs for schizophrenia is on adjunctive therapies. The guidelines anticipate but do not fully explicate clinical trial design issues regarding "broad spectrum" agents, which have both antipsychotic and pro-cognitive effects. CYP-1020 is a new chemical entity that combines dopamine antagonism with GABAergic activity, thus possessing the potential for broad spectrum efficacy. Data from 6-weeks of acute treatment and a 12-week extension of a Phase 2b trial of CYP-1020 in patients with schizophrenia was used to approximate a hybrid clinical trial design, enabling both an acute analysis of antipsychotic efficacy and neurotoxicity of CYP-1020, placebo and risperidone at 6-weeks, and an analysis of pseudospecificity through comparable antipsychotic efficacy but differential cognitive activity of CYP-1020 versus risperidone at 12-weeks.

Methods: The EAGLE (Effective Antipsychosis via GABA Level Enhancement) 6-week study was conducted under a U.S. FDA, IND application at 40 sites in US, Europe, and India. A total of 363 patients with schizophrenia were randomized equally to treatment with low- (10 mg/d; LD) or high- (20-30 mg/d; HD) dose CYP-1020, a positive control using risperidone (2-8 mg/d), or placebo. A subset of patients from the 6-week trial ($N = 60$) were dosed an additional 6-weeks. The study was designed to demonstrate the superiority of high-dose CYP-1020 versus placebo on the total score of the PANSS at 6-weeks. Cognitive impact, as measured by the Brief Assessment of Cognition in Schizophrenia (BACS), was an exploratory endpoint. Safety endpoints included assessment of adverse events, laboratory parameters, and extrapyramidal side effects. *Post hoc* analyses included an examination of changes in PANSS and BACS over 12-weeks.

Results: The ITT LOCF data for the acute (6-week) phase of the trial revealed significant antipsychotic efficacy as measured by change on the PANSS total score for both HD CYP-1020 ($LSM = -23.6$, $SE = 2.45$) and risperidone ($LSM = -26.2$, $SE = 2.45$) versus placebo ($LSM = -14.4$, $SE = 2.40$; $ps < .002$, for both active treatments). There were no statistically significant efficacy differences between HD CYP-1020 and risperidone ($p = .39$). LD CYP-1020 did not separate. Age- and gender-corrected BACS total score changes from baseline were similar among placebo ($LSM = 8.4$,

SE=1.63) risperidone (LSM=8.2, SE=1.46), and LD CYP-1020 patients (LSM=9.2, SE=1.54; all $p>.6$), suggesting no cognitive adverse effects. However, HD CYP-1020 produced an acute cognitive improvement (LSM=12.8, SE=1.58, estimated effect size of 0.5 compared to either placebo or risperidone) versus placebo and risperidone ($p<.03$). An examination of the 60 subjects with 12-week extension data revealed that while HD CYP-1020 produced antipsychotic efficacy similar to that of risperidone at 12-weeks, its pro-cognitive effect was further enhanced over the subsequent 6-weeks, as compared both to the results observed among patients on HD CYP-1020 at 6-weeks ($M=6.4$, $SD=4.3$) as well as patients on risperidone at 12-weeks ($M=3.4$, $SD=11.2$). The correlation between change in PANSS and BACS scores were $<|0.35|$ within the CYP-1020 and risperidone groups for both the acute and chronic phases of the study. There were no clinically relevant changes in the measurements of the ECG, laboratory or vital signs (BP, HR, temp). There were also no clinically relevant changes or AEs of body weight gain, glucose increases, or lipid changes in the CYP-1020 arms.

Discussion: The results of this initial acute/chronic hybrid trial suggest that antipsychotic and pro-cognitive effects can be determined within a single study design. The 20-30 mg/d dose of CYP-1020 in this study was safe and well-tolerated, and appears to have an antipsychotic effect similar to risperidone versus placebo at 6-weeks and a superior pro-cognitive effect versus risperidone at both 6- and 12-weeks. Moving forward, prospectively conducted, appropriately powered acute/chronic hybrid clinical trials may be able to assess antipsychotic efficacy and cognitive functioning in the same study while adequately examining pseudospecificity and neurotoxicity. Such trials could assess patients using both placebo and active controls during the acute treatment phase in schizophrenia, and then follow patients on active therapy over 6-months to fully understand both the antipsychotic and cognitive profiles of broad spectrum agents.

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45. Deep Brain Stimulation for Treatment-Resistant Depression: Follow-Up After 3 to 6 years

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Background: At least 30% of patients with Major Depressive Disorder demonstrate treatment resistance. The high prevalence of treatment resistant depression (TRD) has prompted the investigation of alternative treatment strategies. Among the emerging therapies for

TRD, Deep Brain Stimulation (DBS) represents a promising targeted approach involving bilateral placement of electrodes at specific neuroanatomical sites. Considering the invasive and experimental nature of DBS for TRD, it is important to obtain both short-term and long-term effectiveness and safety data. This report represents an extended follow-up of 20 TRD patients who received DBS to the subcallosal cingulate gyrus- Brodmann Area 25.

Methods: Following the initial 12 month study of DBS, patients were seen annually and at a last follow-up visit to assess depression severity, functional outcomes, and adverse events.

Results: The average response rates 1, 2, and 3 years after DBS were 62.5%, 46.2%, and 75%, respectively. At the last follow-up visit (range 3-6 years), the average response rate was 64.3%. Functional impairment in the areas of physical health as well as social function progressively improved up to the last follow-up visit. No significant adverse events were reported during this follow-up, however, two patients died by suicide during a depressive relapse.

Discussion: These data suggest that in the long-term, DBS remains a safe and effective treatment for TRD. Further trials in a larger sample are needed to confirm these findings.

Disclosure: S. Kennedy: *Part 1*; AstraZeneca, Biovail, Boehringer-Ingelheim, Eli Lilly, Lundbeck, Pfizer, Servier, St. Jude Medical. *Part 4*; AstraZeneca, GlaxoSmithKline, St. Jude Medical, Servier, Lundbeck. P. Giacobbe: *Part 1*; AstraZeneca. *Part 4*; Eli Lilly, St. Jude Medical. S. Rizvi: None. F. Placenza: None. Y. Nishikawa: None. H. Mayberg: *Part 1*; St. Jude Medical. A. Lozano: St. Jude Medical.

46. Does Augmentation of Antidepressant Medications with Psychotherapy Enhance Long-Term Outcome?

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Background: The aims of the present study were to investigate 1) whether a combination of medication plus psychotherapy (MED + THER) would have greater long-term benefits than MED alone and 2) whether MED plus Cognitive Behavioral Analysis System of Psychotherapy (CBASP) would have greater long-term benefits than MED plus Brief Supportive Psychotherapy (BSP) in those chronically depressed patients who exited the randomized phase of the REVAMP (Research Evaluating the Value of Augmenting Medication with Psychotherapy) study.

Methods: We invited all subjects exiting phase 2 of REVAMP to a two year observational follow up. Phase 2 REVAMP randomized subjects who did not fully remit in an algorithmic open trial with antidepressants to 12 weeks of continued MED, MED + CBASP, or MED + BSP (1:2:2). Outcomes (depression symptom severity and functional measures) were assessed every three months.

Results: 326 of 491 eligible subjects (66%) were assessed on one more occasions during the 2 year follow up. Depression symptom scores and functional outcome measures did not change significantly over time, indicating that improvements achieved in phase 2 were maintained. After adjustment for site and propensity for being in the combined medication plus therapy arms, there were no significant differences between MED and MED + THER in depression severity and psychosocial functioning during the observational follow-up. Exploratory analyses found no significant moderating effects of site, clinical status at entry into the follow up (full or partial remission or non-response), and propensity for being in the MED + THER arm. Augmentation with the skill-based psychotherapy CBASP did not produce significantly better long-term outcomes than augmentation of antidepressants with BSP.

Discussion: The addition of psychotherapy, including CBASP, to algorithm-based antidepressant medication did not enhance long term depression and functional outcomes in chronically depressed patients, who had not fully remitted during an initial medication management run in period, over an above MED only.

Disclosure: J. Kocsis: Part 1; Wyeth/Pfizer, Merck. Part 4; Sanofi Aventis, Novartis, CNS Response, Astra Zeneca, Roche, Forest. R. Manber: None.

47. Meditation Improves Depressive Symptoms, Perceived Stress, Coping, Cognition, and Inflammation in Family Dementia Caregivers in a Randomized 8-Week Pilot Study

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Background: This study examined the potential of daily brief meditation practice to improve depressive symptoms, distress, coping, quality of life, cognition, and inflammatory markers in stressed family dementia caregivers in a randomized study of yogic Kirtan kriya compared to relaxation practice.

Methods: Thirty nine family dementia caregivers (mean age 60.3 y.o. (SD=10.2)) were randomized to practice Kirtan Kirya (N=23) compared to listening to the relaxation tapes (N=16) for 20 minutes per day for 8 weeks. The severity of depressive symptoms, resilience, burden, distress, quality of life, suffering, and the severity of care-recipients cognitive and behavioral disturbances were assessed at baseline and over the course of the study. The mean Hamilton Depression Rating Scale (HDRS) score at baseline was 11.6 (SD = 4.1). Cellular expression of the Nuclear Factor kappa Beta (NFkB), a protein complex that has been linked to chronic stress and inflammatory response, was examined in the lymphocytes and monocytes using intranuclear staining and flow cytometry.

Results: The severity of depressive symptoms improved in both groups. However, improvement in the quality of life, cognitive tests (Mini-Mental Status Exam and several executive function tests) ($p < 0.05$) were greater among caregivers practicing meditation compared to the relaxation group, and were accompanied by improvements in sleep, anxiety, and perceived burden. The preliminary data also demonstrated significant decreases in NFkB expression in the meditation group compared to the relaxation group.

Discussion: This small randomized study found that brief daily meditation practice by stressed family dementia caregivers can lead to improved severity of depressive symptoms, distress, coping, cognition, and quality of life compared to relaxation. This improvement is accompanied by decreases in the number of stimulated cells that express NFkB signifying improvement in inflammation. Our results will need to be confirmed in a larger sample.

Disclosure: H. Lavretsky: Part 1; Forest Research Institute.

48. Post Hoc Comparative Effectiveness Study of Paliperidone Palmitate vs Paliperidone ER: an Indirect Comparison from Placebo-Controlled Relapse Prevention Studies

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Background: Paliperidone palmitate is a once-monthly, long-acting injectable atypical antipsychotic for the acute and maintenance treatment of schizophrenia in adults. Currently, no published data on direct comparisons of paliperidone palmitate with oral antipsychotics (eg, paliperidone extended release [ER]) have been reported. This *post hoc* comparative effectiveness research compares the long-term efficacy of maintenance treatment and tolerability of paliperidone palmitate (up to 2 years) with oral paliperidone ER (up to 1 year) in the absence of a head-to-head clinical trial.

Methods: Two nearly identical randomized, double-blind (DB), placebo-controlled schizophrenia relapse prevention trials of paliperidone ER and paliperidone palmitate (Kramer et al, *J Clin Psychopharmacol.* 2007;27:6-14, and Hough et al, *Schizophr Res.* 2010;116:

107-17) were included for analysis based on several nearly similar study criteria: placebo-controlled studies with comparable stabilization and relapse criteria; study designs (ie, run-in/transition, stabilization, DB, and optional open-label extension phases); inclusion/exclusion parameters; and the availability of patient level data. The primary efficacy outcome for each study was the time to first relapse comparing active treatment with placebo in subjects previously stabilized with the active treatment. This *post hoc* analysis was performed on the DB intent-to-treat analysis set: all randomized patients who received at least 1 dose of DB medication and had at least 1 postbaseline efficacy measurement. As the relapse and stabilization criteria differed slightly, an indirect comparison was conducted after adjusting the study populations for stabilization criteria to better match populations between the studies. Assessments included time to relapse, measures of symptom changes and functioning, and treatment-emergent adverse events (TEAEs). Time to relapse between treatment groups was evaluated using Cox proportional hazards models. Demographic and baseline characteristics were summarized using descriptive statistics for stabilization and DB phases. Between-group differences for continuous variables for change scores during the DB phase were assessed using analysis of covariance models. Categorical variables were evaluated using the chi-square or Fisher exact test. Adverse event (AE) rates and corresponding relative risks also were listed.

Results: Approximately 45% of enrolled subjects in both trials were stabilized and randomized to the DB relapse prevention phase. Paliperidone palmitate conferred a statistically significant benefit versus oral paliperidone ER as measured by the time to first relapse (log rank $p < .001$; hazard ratio [HR] = 2.51; 95% CI: 1.46-4.34; $p < .001$). A significant difference was observed between the placebo groups with regard to the time to relapse, favoring subjects who switched from paliperidone palmitate versus those who switched from paliperidone ER (HR = 2.25; 95% CI: 1.59-3.18; $p < .0001$). Those treated with paliperidone palmitate saw virtually no difference between DB baseline and end point in the proportion of patients with mild to no difficulties in functioning (Personal and Social Performance Scale total score > 70 ; 58% vs 59%, respectively) compared with an 11% decrease for paliperidone ER (59% vs 48%, respectively). Overall, TEAE rates for the DB phase were 43% (paliperidone palmitate), 44% (paliperidone palmitate, placebo), 35% (paliperidone ER), and 41% (paliperidone ER, placebo). Similar rates of AEs of interest were observed (paliperidone palmitate vs paliperidone ER, respectively): AEs leading to discontinuation were low (1.6% vs 2.9%); ≥ 1 serious TEAEs (4.2% vs 7.7%); EPS-related AEs (5.7% vs 6.7%); prolactin-related AEs (2.1% vs 2.9%); and weight increase $\geq 7\%$ (23.4% vs 19.6%).

Discussion: This *post hoc* analysis suggests an advantage for paliperidone palmitate in delaying time to relapse when used as maintenance treatment in stable patients with schizophrenia. Similar findings also were observed in comparing the placebo arms during the DB period after withdrawal of active treatment. This may be the result of the difference in pharmacokinetic properties between these agents, with paliperidone palmitate providing therapeutic plasma exposure for a longer period upon discontinuation. Furthermore, despite the long-acting nature of paliperidone palmitate, the AE profile was similar to paliperidone ER. Supported by Ortho-McNeil Janssen Scientific Affairs, LLC.

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49. Achieving and Sustaining Remission in Bipolar I Disorder with Ziprasidone

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Background: Achieving and sustaining remission in bipolar disorder is challenging due to fluctuations in symptomatic stability. Masand et al evaluated remission in subjects with bipolar I disorder receiving aripiprazole in a 26-week study. [1] A recent 6-month, randomized clinical trial reported efficacy of ziprasidone plus either lithium or valproate in bipolar I disorder. [2] Here we conduct *post hoc* analyses of that clinical data to assess rates of symptomatic point remission and sustained remission using several criteria. While the original study was designed to determine time to relapse, our *post hoc* analyses examined the proportion of subjects achieving and sustaining remission. Our hypothesis was that subjects who achieved remission with ziprasidone plus lithium or valproate were more likely to sustain remission than those who were randomized to receive placebo plus lithium or valproate.

Methods: The study comprised an open-label stabilization period of 10 to 16 weeks, during which ziprasidone plus lithium or valproate was administered. Subjects who achieved stability for 8 consecutive weeks on the adjunctive regimen were randomized in the double-blind phase to ziprasidone or placebo, in combination with lithium or valproate and followed for 6 months. We conducted *post hoc* analyses to investigate 2 definitions of remission at the start of the double-blind phase: Mania Rating Scale (MRS) score ≤ 7 or MRS ≤ 7 and Montgomery-Åsberg Depression Rating Scale (MADRS) score 8 weeks and for 24 weeks.

Results: At the end of the open-label stabilization phase, 238 subjects (53%) receiving ziprasidone plus lithium or valproate achieved remission and were randomized in the double blind phase. The rates of remission during the double-blind phase, defined as MRS ≤ 7 , at weeks 8, 16, and 24 were: $n = 89$ (70.1%), $n = 87$ (68.5%), and $n = 76$ (59.8%) for ziprasidone plus lithium or valproate, compared with 68 (61.3%), $n = 53$ (47.7%), and $n = 46$ (41.4%) for placebo plus lithium or valproate ($p = 0.20$, $p = 0.0003$, and $p = 0.004$ for weeks 8, 16, and 24, respectively). When remission was defined as MRS ≤ 7 and MADRS ≤ 10 , the rates of remission at weeks 8, 16, and 24 were: $n = 70$ (55.1%), $n = 64$ (50.4%), and $n = 61$ (48.0%) for ziprasidone plus lithium or valproate, compared with $n = 62$ (55.9%), $n = 48$ (43.2%), and $n = 41$ (36.9%) respectively, for placebo plus lithium or valproate ($p = 0.73$, $p = 0.04$, and $p = 0.04$ for weeks 8, 16, and 24, respectively). For remission defined as MRS ≤ 7 sustained for at least 8 weeks, remission rates for ziprasidone treatment at 8, 16, and 24 weeks were $n = 66$ (52.0%), $n = 72$ (56.7%) and $n = 72$ (56.7%), respectively, compared with $n = 50$ (45.0%), $n = 51$ (45.9%) and $n = 42$ (37.8%), respectively for placebo ($p = 0.27$, $p = 0.07$, and $p = 0.003$ for weeks 8, 16, and 24, respectively).

Discussion: Our *post hoc* analyses determined that in a study designed to investigate the maintenance effects of ziprasidone plus lithium or valproate in bipolar I disorder, of those subjects who achieved remission, most maintained remission at weeks 8, 16, and 24. This indicates that not only is ziprasidone effective in preventing relapse in bipolar I disorder but also leads to sustained remission. References: (1) Masand, P.S., Eudicone, J., Pikalov, A., McQuade, R.D., Marcus, R.N., Vester-Blokland, E., Carlson, B.X., 2008 Criteria for defining symptomatic and sustained remission in bipolar I disorder: a post-hoc analysis of a 26-week aripiprazole study (study CN138-010). *Psychopharmacol Bull* 41, 12-23. (2) Bowden, C.L., Vieta, E., Ice, K., Schwartz, J.H., Wang, P.P., Versavel, M., 2010. Ziprasidone plus a mood stabilizer in subjects with bipolar I disorder: a 6-month, randomized, placebo-controlled, double-blind trial. *Clin Psychiatry* 71, 130-137. This study was supported by Pfizer Inc.

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50. Including a Priori Knowledge to Define Under-Performing Recruiting Centres Could Help to Improve “Signal Detection” in Antidepressant Multicenter Trials: a Clinical Trial Simulation Study

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Background: Multicenter randomized clinical trials (RCTs) in depression are relatively inefficient. Failure to differentiate known effective antidepressant drug treatments from placebo is accounted in ~50% of trials. The main reason for this failure is thought to be an uncontrolled placebo response. To better understand the phenomenon, a “typical” placebo response trajectory in RCT for paroxetine was defined by recruitment center using a large database (Gomeni & Pich 2006). A Bayesian approach was then used to quantify the capacity of a centre to act as “signal detector” for treatment effects by focusing only on their placebo response at study-end. We found that recruitment centres of a given RCT widely differ in their placebo response scores, possibly related to the different management and/or recruitment of subjects. This happens in spite of the training sessions to the staff during the Trial Investigator Meetings regarding GCP. Currently, no information concerning centre performance capacity is considered during randomization. Therefore, in any novel RCT there is a risk is to include centres that excessively either mismanage expectations of subjects or excessively misdiagnosed them, leading to a silent bias. We propose that (1) when these excessively underperforming centres are over-represented within a given multicentric RCT the probability of failure will be high. We also propose (2) that if reasonable and clinically relevant criteria derived from the *a priori* knowledge of the typical placebo response are available, those criteria should be used to improve the RCT outcome and included in the Study Protocol. In other words, centres that do not produce the appropriate placebo response at study-end (i.e., within a Bayesian likelihood range) should be disqualified and not included in the “informative population” analysis aimed to signal detection. This process should result in reducing the “silent bias” introduced by the lack of control of the centre performance capacity before study-start. Here clinical trial modelling & simulation was used to conceptually test these 2 hypothesis.

Methods: Data were derived from GlaxoSmithKline clinical trial register [<http://ctr.gsk.co.uk/medicinelist.asp>]. Data from 3 randomized, double-blind, placebo controlled, parallel group studies for treatment of Major Depressive Disorders, with HAMD-17 total (HAMD) as primary efficacy measurement and with study duration of 8 weeks (study 810, 448 and 449), consisting of a total of 77 centres and 821 subjects were considered. Treatment responses were defined by the time-varying scores of the HAMD and analysed using a mixed Weibull/linear equation where A is the baseline HAMD score, t_d is the time corresponding to 63.2% of the maximal change from baseline, b is the shape or sigmoidicity factor, and h_{rec} is the remission rate. Clinical trial simulation was used to assess how different distributions of placebo response in the different centers may affect the detection of clinical effect and the success of RCTs. Treatment response in each center was simulated by selecting the set of individual subject trajectories, with HAMD scores simultaneously falling within the center-specific 95% confidence intervals of HAMD at baseline and at week 8. For the study 810, several trial simulation scenarios were evaluated with different numbers of under-performing centers.

Under-performing centers were qualified as those centers with placebo arm with HAMD > 10 (partial remission) or higher than 10% of the baseline HAMD scores when assessed at study-end (Merlo Pich et al. 2010). For each scenario 50 simulated trials were performed. A change in HAMD score lower than 3 indicated a failed RCT.

Results: Simulations of the expected outcomes of study 810 showed detectable magnitude of treatment effect that varies from 4.1 to 1.23 points when the median placebo varied from 15.5 to 9.8 points. Initially RCTs with 25mg paroxetine treatment were simulated: When under-performing centers where above 25% of the total centers per RCT, the probability to obtain a failed trials was over 90%. This effect was significantly attenuated by reducing the number of under-performing centers per study, stabilizing the response over effective dose range. Other simulations are currently in progress.

Discussion: Clinical trial simulation was used to better understand the possible impact of highly variable placebo response per recruitment centres in antidepressant trials. Practical applications still await.

Disclosure: E. Merlo Pich: Part 5; GlaxoSmithKline. R. Gomeni: GlaxoSmithKline.

51. A Randomized, Double-Blind Study Comparing LY2216684, a Selective Norepinephrine Reuptake Inhibitor and Placebo in the Treatment of Major Depressive Disorder

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Background: The efficacy and safety of LY2216684, a selective norepinephrine reuptake inhibitor, was studied in adult patients with major depressive disorder (MDD).

Methods: The study design was a double-blind, placebo-controlled, flexible dose of LY2216684 6-18 mg once daily compared with placebo for 10 weeks of acute therapy followed by a 1 year open-label extension (dose blinded) (not reported here). Primary inclusion criteria were a diagnosis of MDD and Hamilton Depression Rating Scale 17 items total score ≥ 18 . The primary efficacy measure was the Montgomery-Asberg Depression Rating Scale (MADRS). Key secondary measures were Sheehan Disability Scale (SDS) and the patient reported Fatigue Associated with Depression Scale (FASD).

Results: Of 495 randomized patients, 250 received LY2216684 and 245 received placebo. LY2216684-treated patients significantly improved on mean MADRS total score compared with placebo-treated patients (-13.3 vs -9.8, respectively, $P \leq .001$). Estimated probability of response (MADRS total score improvement $\geq 50\%$ from baseline) was significantly higher for LY2216684 compared with placebo treatment (43.3% vs 27.7%, respectively, $P \leq .001$), as was estimated probability of remission, defined as MADRS total score ≤ 10 (25.5% vs 17.8%, respectively, $P \leq .05$). LY2216684 was associated with significantly greater improvement on SDS global functioning impairment score, FASD average, and FASD subscale scores compared with placebo. Discontinuation rate due to adverse events was 9.6% for LY2216684-treated patients compared with 1.6% for placebo-treated patients ($P < .001$). Compared with placebo, LY2216684 treatment was associated with significant increases in mean systolic (3 mm Hg) and diastolic (4 mm Hg) blood pressure and pulse (10 bpm).

Discussion: LY2216684 6-18 mg demonstrated significant efficacy and was generally well tolerated in the treatment of MDD.

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52. Oxytocin Treatment Improves Social Cognition and Reduces Psychotic Symptoms in Schizophrenia

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Background: Social dysfunction is the most common symptom as well as the primary cause of disability in schizophrenia and remains unresponsive to currently available antipsychotic medications. Social impairment has been strongly linked to deficits in social cognition, mental capabilities that facilitate social decisions and behavior. Decades of animal research have established that oxytocin (OT) has many pro-social effects. Preclinical investigations have demonstrated that OT has antipsychotic-like efficacy. In recent human studies, acute intranasal OT administration increased interpersonal trust, eye contact, performance on tests of face emotion recognition and theory of mind (inferring the thoughts and feelings of others) as well as social reciprocity in normal and autistic subjects. Feifel et al recently reported that OT treatment of subjects with schizophrenia for 3 wks decreased PANSS scores. We hypothesized that intranasal OT treatment of patients with schizophrenia may improve social cognition as well as reduce psychotic symptoms.

Methods: We conducted a randomized, double blind, placebo-controlled pilot study in subjects with paranoid or undifferentiated schizophrenia for 1 yr or more and baseline PANSS total scores of 60 or more comparing the effects of 14 days of twice daily intranasal OT (24 IU/dose, N = 8) vs. placebo (N = 6) administration on the following outcomes: a) PANSS total and subscale scores; b) *a priori* selected items on the PANSS, c) subject self-ratings on the Paranoia Scale and d) a battery of social cognition tests. Subjects' symptoms and psychotropic medication regimens were stable for >1 month prior to the treatment trial and medication doses remained unchanged during the study period.

Results: The sample was comprised of 12 men and 2 women; 8 white and 6 African-American. There were no significant demographic or psychiatric history differences between the treatment groups and no significant changes in laboratory tests or vital signs during the treatment period (assessed at multiple time points). Weight increased significantly in the placebo group but not the OT group. No adverse events occurred and no side effects were reported or observed. Baseline measures did not differ between treatment groups except the OT group had lower face trustworthiness ratings. The OT group had significant reductions from baseline to treatment day 14 in PANSS total scores ($t = 3.18$, $p = .015$), positive symptom subscale scores ($t = 2.73$, $p = .03$), general symptom subscale scores ($t = 2.87$, $p = .02$), suspiciousness item scores ($t = 2.97$, $p = .02$), anxiety item scores ($t = 3.06$, $p < .02$) and Paranoia Scale scores ($t = 2.67$, $p = .03$), as well as significant improvements in accurate identification of 2nd order false belief in the Brüne Theory of Mind task ($t = -3.42$, $p = .01$). In addition, OT recipients showed a weak trend toward rating untrustworthy faces (faces rated by a normative sample as untrustworthy) as less untrustworthy ($t = 1.66$, $p = .14$). The mean scores on an *a priori* subset of PANSS items relevant to social functioning (P6 [suspiciousness/persecutory], P7 [hostility], N4 [passive/apathetic social withdrawal], G8 [uncooperativeness] and G16 [active social avoidance]) decreased more than any other variable ($t = 3.99$, $p = .005$). The placebo group had no significant or trend level changes from baseline to treatment day 14 on any of these measures. ANCOVAs controlling for baseline revealed that the OT compared to the placebo group had significantly greater declines in PANSS anxiety item scores ($F = 6.6$, $p < .03$) and mean social function relevant item scores ($F = 5.6$, $p < .04$) and trends for greater improvement in all other variables described above except for trustworthy ratings of faces (p values from 0.6-0.19).

Discussion: This is the first study to demonstrate that OT treatment improves social cognition in schizophrenia and replicates the findings of Feifel et al that OT decreases psychotic symptoms. Our results raise several pressing questions. 1) Will a longer period of OT treatment

improve social functioning and further reduce psychotic symptoms? 2) Is OT an effective monotherapy or is it only efficacious as an augmentor of antipsychotic medications? 3) Would concomitant OT treatment improve outcomes of psychosocial interventions? 4) Can OT treatment stave off the social and functional deterioration observed early in the course of schizophrenia (first episode) or even prior to the development of the illness (prodrome)? Validation of our findings would justify new lines of investigation into the pathophysiology of schizophrenia focusing on the role of central OT systems.

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53. Association Between Bipolar Spectrum Features and Treatment Outcomes in Outpatients with Major Depressive Disorder

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Background: Patients with major depressive disorder and pretreatment clinical features suggestive of bipolar disorder, or bipolar spectrum features, may have poorer treatment outcomes. This assertion has rarely been examined empirically.

Methods: We examined individuals participating in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, an effectiveness study conducted at primary and specialty care centers in the United States. Putative bipolar spectrum features, including items on the mania and psychosis subscales of the Psychiatric Diagnosis Screening Questionnaire, were examined for association with treatment outcomes.

Results: Of 4,041 subjects who entered the study, 1,198 (30.0%) endorsed at least one item on the psychosis scale, and 1,524 (38.1%) described at least one recent manic/hypomanic-like symptom. Irritability and psychotic-like symptoms at entry were significantly associated with poorer outcomes across up to 4 treatment levels, as were shorter episodes and some neurovegetative symptoms of depression. Other indicators of bipolar diathesis including recent manic-like symptoms and family history of bipolar disorder, as well as summary measures of bipolar spectrum features, were not associated with treatment resistance.

Discussion: These data provide no support for the hypothesis that unrecognized 'bipolar spectrum' illness contributes substantially to antidepressant treatment resistance.

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Somerset, Takeda, Tetragenex, TransForm, Transcept, Vanda Pharmaceuticals, EnVivo Pharmaceuticals, Clintara, Covidien, RCT Logic, CeNeRx BioPharma, GenOmind, Prexa Pharmaceuticals, Rexahn Pharmaceuticals, Sepracor. *Part 4*; Eli Lilly, GlaxoSmithKline, Bristol-Myers Squibb, PamLab, Sanofi-Aventis, Pfizer, AstraZeneca, Johnson&Johnson, Novartis, Healthcare Technologies, BrainCells.

54. The Relationship of Components of Delusionality to Treatment Outcome in a Placebo-Controlled Trial of Fluoxetine for Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) - a distressing or impairing preoccupation with nonexistent or slight defects in physical appearance - is a severe and relatively common disorder whose treatment has been minimally studied. Appearance beliefs (e.g., "I am deformed") span a range of insight, from excellent to absent insight (i.e., delusional beliefs). We previously reported results from the only placebo-controlled pharmacotherapy study of BDD which found that fluoxetine was more efficacious than placebo, and that BDD symptoms of delusional patients were as likely as symptoms of non-delusional patients to respond to fluoxetine monotherapy. The latter finding is consistent with other SRI studies of BDD. In addition, treatment responders had greater decreases in delusionality than non-responders, but delusionality did not decrease significantly more with fluoxetine than placebo. Here we present new post-hoc analyses of one of the fluoxetine study's exploratory aims, in which we examine individual components of delusionality of BDD beliefs in relation to treatment outcome. Delusionality is a multidimensional construct, and the relationship between individual components of delusionality and treatment outcome may potentially inform patient care. To our knowledge, no prior study has examined this.

Methods: After 1 week of single-blind placebo, 67 patients with DSM-IV BDD (including delusional BDD) were randomized to 12 weeks of double-blind treatment with fluoxetine or placebo. The reliable and valid BDD-YBOCS, the primary outcome measure, assessed change in BDD symptoms. The reliable and valid Brown Assessment of Beliefs Scale (BABS) assessed global delusionality and the following components of delusionality: 1) conviction the belief is accurate, 2) perception of others' views about the belief's accuracy, 3) explanation for differing views (between self and others), 4) fixity (whether the person could be convinced the belief is wrong), 5) attempt to disprove the belief, 6) recognition that the belief has a psychiatric/psychological cause, and 7) ideas/delusions of reference. Generalized linear mixed modeling (GLMM) tested whether group (fluoxetine vs. placebo) and response to treatment (30% or greater improvement in BDD-YBOCS score) were related to change in components of delusionality during treatment. GLMM also examined components of delusionality at baseline as predictors of improvement in BDD symptoms.

Results: The mean baseline BABS score was 18.0 ± 4.4 (poor insight to delusional range); 42% of patients had delusional BDD beliefs and 58% had nondelusional BDD beliefs. Regarding change in delusionality of BDD beliefs with treatment, there was a significant response x time interaction for all components of delusionality ($p < .001 - .004$), except for BDD-related ideas/delusions of reference, such that treatment responders had greater decreases in components of delusionality than non-responders. However, none of the delusionality components decreased significantly more with fluoxetine than placebo treatment. Ideas/delusions of reference did not significantly change during treatment regardless of treatment group or response. In GLMM analyses of baseline BABS items as predictors of change in BDD symptoms, the baseline BABS conviction item showed a significant three-way interaction of time x group x baseline level of conviction ($p = .046$). In the placebo group, subjects with lower baseline conviction scores had greater improvement in BDD symptoms than subjects with higher baseline levels of conviction. However, in the

fluoxetine group, subjects had similar decreases in BDD symptoms regardless of baseline conviction level. A time x baseline level of fixity interaction ($p = .016$) and a time x baseline level of explanation of differing views interaction ($p = .041$) were found; subjects treated with fluoxetine or placebo who had lower scores on baseline fixity and explanation of differing views had significantly greater improvement in BDD symptoms than subjects with higher baseline scores on these items. The other BABS items did not significantly predict change in BDD symptoms in either treatment group.

Discussion: All components of delusional ideation improved in treatment responders, regardless of treatment type. Baseline conviction, fixity, and explanation of differing views were significantly related to improvement in BDD symptoms. These post-hoc exploratory analyses may inform future research on the relationship of delusional ideation to treatment outcome, which warrants further study in pharmacotherapy and psychosocial intervention studies.

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55. A Pooled Analysis of the Effects of Asenapine on the Persistent Negative Symptoms of Schizophrenia

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Background: Treatment with both asenapine and olanzapine reduced negative symptoms, measured by change from baseline on the 16-item Negative Symptom Assessment (NSA-16) scale, in adults with persistent negative symptoms of schizophrenia in 2 identically-designed double-blind, randomized 26-week core studies conducted in the Eastern (EH) or Western Hemisphere (WH) and in their respective 26-week blinded extensions. A significant treatment difference was observed in the WH extension only, in which asenapine was found to be superior to olanzapine at week 52 in participants with ≥ 6 months of exposure. To more fully explore the effects of asenapine, we conducted *post hoc* analyses using pooled data from these 4 trials.

Methods: Each of the 2 core studies and their respective extensions were double-blind, olanzapine-controlled, flexible-dose trials conducted in the WH (clinical trials registry: NCT00145496, NCT00174265) or EH (NCT00212836, NCT00265343). Core study participants were randomly assigned to sublingual asenapine (5 mg twice daily [BID] during week 1; 5 or 10 mg BID thereafter) or oral olanzapine (10 mg once daily [QD] during week 1; 5-20 mg QD thereafter), with matching sublingual or oral placebo used to maintain blinding. Participants entering the extensions were maintained on the treatment regimen used at the end of each core study, with no rerandomization. Efficacy endpoints, changes in NSA-16 total score from core study baseline to endpoint of the core studies (treatment week 26) or from core study baseline to endpoint of the extension studies (treatment week 52), were assessed using a mixed model for repeated measures analysis on the intent-to-treat populations among the pooled data sets.

Results: A total of 949 participants were randomized to treatment in the 2 core studies (asenapine, $n = 485$; olanzapine, $n = 464$). Of the 613 participants (asenapine, $n = 277$; olanzapine, $n = 336$) who completed

26 weeks of treatment, 502 (asenapine, $n = 220$; olanzapine, $n = 282$) entered the 26-week extensions and 412 (asenapine, $n = 170$; olanzapine, $n = 242$) completed an additional 26 weeks of treatment. The incidence of discontinuation due to lack of therapeutic effect (defined as worsening of schizophrenia as an adverse event plus lack of efficacy) was significantly greater for asenapine versus olanzapine for the first 26 weeks among all treated participants entering the core studies (13.6% vs 7.3%, $p = 0.0016$) and among all treated participants entering the extensions (5.5% vs 2.1%, $p = 0.0458$). After 26 weeks of treatment, the least squares (LS) mean \pm SE change from core study baseline in NSA-16 total score did not significantly differ between asenapine and olanzapine among all participants who entered the core studies (-11.1 ± 0.6 with asenapine vs -11.2 ± 0.6 with olanzapine; $p = 0.9457$) or among participants who entered the extension (-13.1 ± 0.7 with asenapine vs -12.2 ± 0.6 with olanzapine; $p = 0.3710$). At week 52 of treatment, the LS mean \pm SE change from core study baseline in NSA-16 total score was significantly greater with asenapine among all participants who entered the core studies (-14.6 ± 0.8 vs -12.6 ± 0.7 with olanzapine; $p = 0.0497$) and among all participants who entered the extension studies (-16.5 ± 0.9 vs -13.6 ± 0.7 with olanzapine; $p = 0.0083$).

Discussion: These pooled *post hoc* analyses indicate that treatment with both asenapine and olanzapine reduced the negative symptoms of schizophrenia in adults with persistent negative symptoms. Statistical superiority of asenapine was observed at week 52 (the extension study primary endpoint) but not at week 26 (the core study primary endpoint). However, these results need to be interpreted in view of the fact that a large portion of participants did not enter the extension studies and those that did enter the extension continued treatment without rerandomization.

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56. Treatment of Neurotropic Infectious Agents to Alleviate Cognitive Deficits in Schizophrenia: A Test of Concept Randomized Double Blind Placebo Controlled Trial

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Background: Cognitive impairments contribute significantly to poor long term outcome of schizophrenia (SZ) and respond minimally to antipsychotics. A potentially treatable factor that has shown replicable association with cognitive impairments and prefrontal cortical grey matter among subjects with SZ is exposure to neurotropic herpes simplex virus, subtype 1 (HSV1). Further, HSV1 exposed SZ subjects were noted to have longitudinal changes in cognitive impairments along with progressive grey matter loss compared to those not exposed. These observations were made on individuals without a history or evidence of encephalitis suggesting that asymptomatic exposure to HSV1 may be of significance. Since effective medications are available to treat herpes infections, we conducted a hypothesis-driven test of concept trial of Valacyclovir (VAV) add-on treatment for early course SZ subjects.

Methods: We randomized 24 HSV1 positive SZ subjects with a mean duration of illness of 5.0 ± 3.34 years to receive VAV + antipsychotics (AP) or placebo (PL) + AP for 18 weeks (12 subjects in the PL + AP and 12 in the VAV + AP group). Majority of the subjects completed study. Subjects were on stable doses of atypical antipsychotics. Antipsychotics were not switched and no new medications were added during the study. Substance use and adherence to both study and prescribed medications were monitored at regular study follow ups (weeks 2, 4, 6, 8, 12, 16 & 18). VAV was started at 1 g PO BID for 2 weeks and then increased to 1.5 g PO BID since this dose was found to provide the same concentration of VAV in the brain as receiving intravenous therapeutic acyclovir. At each visit, subjects were administered the Positive and Negative Symptom Scale (PANSS) and side effect scales. Computerized Neurocognitive Battery (CNB) was used to evaluate cognitive performance at baseline and follow up that included working memory, spatial memory, attention, spatial processing, verbal memory, and face memory, and emotion processing. Intent-to-treat analysis using linear mixed effects models that included all randomized patients were used to examine differential changes in cognition and psychopathology over the duration of the study between those assigned to VAV + AP or PL + AP, accounting for placebo response. For the CNB scores, we examined reaction time and accuracy separately.

Results: Subjects in VAV + AP group showed modest improvements in accuracy on n-back and delayed visual learning, and response time on verbal working memory compared to PL + AP. Overall accuracy of n-back showed a trend toward improvement ($t = 2.19$, $p = 0.06$); the differences primarily contributed by improved accuracy in 2-back test ($t = 2.42$, $p = 0.045$) but not by 0-back ($p = 0.12$) or 1-back ($p = 0.52$). The delayed visual learning accuracy ($p = 0.011$) and the reaction time for verbal working memory ($t = 3.28$, $p = 0.0002$) showed improvement in the VAV + AP group. The effect size (Cohen's d) was 0.73 for n-back accuracy, 1.2 for visual learning and 1.5 for verbal memory reaction time. We did not observe changes in psychotic symptom severity between these groups.

Discussions: This is the first study to suggest that supplemental treatment with VAV may be beneficial for cognitive impairments in early course SZ subjects but not positive or negative symptoms. If the results of the study are replicated on a larger cohort, this approach could provide a novel strategy to treat a major therapeutic challenge in SZ.

Disclosure: K. Prasad: None. S. Eack: None. M. Keshavan: None. R. Yolken: None. V. Nimgaonkar: None.

57. Cognitive, Neuroendocrine, and Symptom Change in PTSD Treatment of OEF/OIF Veterans

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Background: While effective psychotherapies for the treatment of PTSD exist (Foa et al, 2008), the biological and cognitive mechanisms involved in effective treatment have received little empirical attention. The current study provides an examination of changes in cortisol and changes in trauma related cognitions in relation to PTSD symptoms during PTSD treatment.

Methods: Veterans and active duty Service Members ($N = 36$) were randomly assigned to receive 10-12 weekly sessions of either Prolonged Exposure Therapy (PE) or Present Centered Therapy (PCT). Twenty two patients completed treatment and 14 dropped out or were withdrawn. Pre, mid, and post treatment data on these returnees will be presented. Several different challenge tasks, targeting the fear response system, have been included to examine processes involved in treatment change. The current analyses will examine cortisol response to awakening and cognitive changes over treatment.

Results: Returnee average age was 32 ($SD = 7.6$). Fifty-five percent were married. Most served in Iraq (86%), but a significant minority serve in Afghanistan (22%; some served in both). Returnees report moderately severe PTSD at pre treatment on the Clinician Administered PTSD Scale ($M = 78.6$, $SD = 11.7$). Preliminary results were examined on the first 15 completers. To examine whether changes in cortisol response to awakening were related to treatment response or treatment condition, two repeated measures ANOVAs were conducted. PE but not PCT leads to a normalization of cortisol awakening response among those veterans receiving PE such that the PE group has a higher AUC than the PCT group, $F(1,14) = 11.1$, $p = .005$. Further, responders demonstrated a normalization of cortisol response to awakening such that at post treatment the difference in cortisol response to awakening approaches significance, $t(14) = -1.88$, $p = .08$. In order to examine the relationships between change in each of these factors, standardized residual gain scores were calculated for each variable (cognition, cortisol RTA, PTSD severity score) and correlational analyses conducted. Changes in negative thoughts about the self, the world, and self blame correlated highly with reductions in PTSD severity [Self, $r(28) = .70$, $p < .0001$; World, $r(28) = .38$, $p = .04$; self-blame, $r(28) = .52$, $p = .005$]. The poster will include mediational analyses (Baron & Kenny, 1986) examining the potential mediation of the relationship between cognitive change and PTSD change by change in cortisol response to awakening. All data is collected and final cortisol assays are being conducted. Poster will present final cortisol data.

Discussion: Change in trauma related cognitions are related to change in PTSD symptoms accounting for about half of the variance in reduction of PTSD with treatment. Changes in cortisol response to awakening are related to response to PTSD treatment with a normalization of cortisol response to awakening occurring over the course of effective PTSD treatment. Implications of these findings for the identification of potential treatment mechanisms will be discussed.

Disclosure: S. Rauch: None. A. King: None. B. Rothbaum: None. E. Smith: None. I. Liberzon: None.

58. Results of a Proof-of-Concept, Dose-Finding, Double-blind, Placebo-Controlled Study of Serdaxin in Subjects with Major Depressive Disorder

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Background: Serdaxin (active ingredient clavulanic acid) is a small molecule that increases both serotonin and dopamine metabolites in preclinical models of antidepressant activity. Unlike most commonly used antidepressants, Serdaxin does not bind to the serotonin transporter or other receptors associated with monoamine activity and does not appear to affect serotonin reuptake. In microdialysis studies with rats Serdaxin increases the release of dopamine and serotonin metabolites. Due to Serdaxin's action in preclinical models, a proof of concept study in humans was conducted to determine whether Serdaxin would have antidepressant efficacy in a clinical population.

Methods: This was a multi-center, randomized, double blind, placebo controlled, parallel group study in depressed subjects (defined by DSM-IV criteria) aged 18-65 years, who had HAM-D-17 baseline score ≥ 20 . Eligible subjects were randomly assigned to receive double-blind treatment with Serdaxin (5, 10 or 15 mg/BID) or placebo for 8 weeks. Change from baseline in MADRS total score was the primary endpoint with HAM-D, Global Clinical Impression and Quick Inventory of Depressive Symptomatology as secondary endpoints after 8 weeks of treatment using LOCF for missing data.

Results: A total of 77 subjects were randomized. Completion rates for the Serdaxin arms were high (85.7, 64.7, 94.1% respectively), as compared to only 41% in the placebo arm. The mean changes from baseline to endpoint in MADRS scores were -46.0%, -37.9% and -1.4% for 5, 10 and 15 mg/BID Serdaxin, respectively, compared to 43.1% for placebo using LOCF for missing data. Among the subset of subjects

with relatively severe depression (baseline MADRS ≥ 29 ; $n=28$), MADRS scores improved 55.6% with 5 mg/BID Serdaxin but only 34% with placebo ($p=0.041$). In an analysis of responders ($\geq 50\%$ improvement from baseline in MADRS scores), 64.3% of severely depressed subjects treated with 5 mg/BID Serdaxin responded compared to 28.6% of placebo treated. All doses of Serdaxin were well tolerated, with only 2-4% of patients discontinuing prematurely due to adverse events.

Discussion: Although this study did not demonstrate efficacy for Serdaxin on the primary analysis, a *ad-hoc* analysis of outcomes of patients with more severe depressive symptoms suggested that the 5 mg/BID dose may have clinically significant antidepressant effects. Serdaxin was well tolerated at all doses. These findings suggest that further studies of Serdaxin at lower doses are warranted.

Disclosure: R. Riesenberger: None. J. Rosenthal: None. L. Moldauer: Part 1; INC Research. C. Peterson: Rexahn Pharmaceuticals, Inc.

59. The Importance of Quality in Post-baseline Assessments in CNS Clinical Trials

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Background: The high failure rate of psychiatric clinical trials is well-documented. Contributors to this include baseline score inflation, functional unblinding, rater drift and expectancy bias. When enrollment pressures cause inflated baseline severity scores or inaccurate diagnoses, inappropriate subjects can be enrolled in a study. Methods have been developed to address baseline score inflation as a source of error, and an increasing number of studies now include processes for ensuring that the right subjects are entered into a trial, such as the use of independent blinded raters, or monitoring of site rater assessments. There is less recognition of post-baseline factors that can affect outcomes. Functional unblinding can occur when a test drug or active comparator is associated with adverse events that unblind raters as to which subjects are in which arm. This can be avoided by separating efficacy raters from assessing adverse events, and by using a different interviewer at each visit so raters do not notice side effects over time, such as weight gain. Rater drift increases the variability of ratings, adding to noise in a trial that can obscure signal detection. Continuous calibration of raters is necessary to avoid rater drift. Finally, expectancy bias can greatly increase placebo effect, also obscuring or diminishing detection of a drug-placebo difference. Using a different rater at each visit who is independent from the subjects' sites and therefore does not spend time with subjects, avoids expectancy bias. This poster will address the extent to which there is a need for centralized independent blinded assessments after subjects are randomized.

Methods: Studies with ratings completed by both site raters and blinded remote raters can be evaluated to see how critical are continued blinding and continuous calibration throughout a trial independent of subject selection. In a clinical trial of acute schizophrenia, remote blinded raters conducted the PANSS and site raters used the BPRS on the same 313 subjects, within 1-2 days of the blinded raters' assessments. The remote blinded raters were independent from the sites, and carefully and continuously calibrated with each other throughout the study. A recently-completed study of 259 subjects with Parkinson's psychosis included blinded independent central ratings in the US, and traditional site ratings in the OUS sites. Both sets of raters used the hallucinations and delusions subscales of the SAPS as the primary outcome measure. Site physicians across geographies (including US) selected the subjects, so the main difference in methods was the use of blinded central raters in the US for post-subject-selection visits. Finally, in a negative clinical trial of GAD we can examine the effect of blinded calibrated ratings on placebo response. Both blinded central raters and site raters administered the SIGH-A to 122 subjects who had been admitted to the study based on site raters' evaluations.

Results: In the schizophrenia trial, the independent blinded raters differentiated subjects on placebo, the active comparator, and one of two test arms, throughout the study. Site raters distinguished placebo from the active comparator, but did not separate either of the two test arms. In the Parkinson's psychosis study, the test drug was separated from placebo by the central raters (significantly at week 2 and trend at week 6), but no signal was detected by the site raters OUS using the same study design. Finally, the GAD trial shows that the remote blinded raters had a lower placebo response rate independent of subject selection; in all subjects as well as in the cohorts that the blinded raters would have excluded, and those that both cohorts of raters agreed should be included.

Discussion: Data from several studies support the importance of the accuracy of assessments after subjects have been selected. Data presented indicate that precision of ratings beyond baseline can increase the sensitivity of findings in a clinical trial, decrease placebo response rates and potentially eliminate Type II errors (false negatives). Blinding of raters to study protocol and visit number can decrease or eliminate expectancy bias and functional unblinding in post-baseline measurements. Independence from subjects' sites reduces expectancy bias by reducing the amount of time rating staff spends with subjects, and decreasing the development of ongoing relationships between raters and subjects. Lack of rater calibration or rater drift, even with experienced raters, can interfere with the final measurement of drug-placebo differences and can be minimized only through continuous calibration of raters as a cohort.

Disclosure: J. Williams: Part 1; MedAvante, Inc. Part 2; MedAvante, Inc. Part 3; MedAvante, Inc. Part 5; MedAvante, Inc. K. Kobak: Part 1; MedAvante, Inc., Center for Psychological Consultation. Part 2; MedAvante, Inc., Center for Psychological Consulting. Part 3; MedAvante, Inc., Center for Psychological Consulting. Part 4; SBIR Grant from NIMH. M. Detke: Part 1; MedAvante, Inc., Eli Lilly. Part 2; MedAvante, Inc., Eli Lilly. Part 3; MedAvante, Inc., Eli Lilly. Part 5; MedAvante, Inc., Eli Lilly.

60. A Brief Cognitive Assessment Tool for Schizophrenia (B-CATS): Scale Validation

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Background: Schizophrenia is marked by a profound global cognitive impairment that contributes more to chronic disability and unemployment than do positive and negative symptoms. New treatments are being developed for cognition in schizophrenia, but clinicians are limited in their ability to assess cognition due to a lack of easily administered and interpretable instruments. We previously developed a very brief battery by selecting existing cognitive tests that together generate a summary score representing global cognitive function (Hurford et al; Schizophrenia Bulletin; 2009). The Brief Cognitive Assessment Tool for Schizophrenia (B-CATS) was developed specifically to provide clinicians with a way to assess global cognition in their patients with schizophrenia. Here, we report the results of a validity study comparing B-CATS to a larger neurocognitive battery, the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery.

Methods: Outpatients with schizophrenia as diagnosed by SCID ($N=91$) were administered the MATRICS battery and the B-CATS at two time points separated by 1 month. They were also administered the UCSD Performance-Based Skills Assessment-Brief (UPSA-B), a measure of functional capacity.

Results: Several possible B-CATS variants were examined for reliability and correlation with the MATRICS battery. The final version of the B-CATS has an administration time of approximately 10 minutes. It demonstrates good test-retest reliability ($r=0.792$, $ICC=0.789$) and inter-item consistency (Cronbach's $\alpha=0.729$). It correlates 0.786 ($p<0.01$) with the MATRICS battery, and 0.657 with the MATRICS battery excluding B-CATS tests ($p<0.01$). The final B-CATS and the

MATRICES battery correlate with the UPSA-B at 0.509 and 0.579 respectively.

Discussion: Prior research (e.g. Keefe et al; Neuropsychopharmacology; 2006) has demonstrated that a single factor of cognitive function best explains the deficit pattern of schizophrenia. Therefore, a test that generates a global summary score could provide clinicians with meaningful data regarding their patients' cognitive status. The 10 minute B-CATS correlates highly with the "gold standard" neurocognitive battery that has an administration time of over 60 minutes. Both measures correlate moderately with a measure of functional capacity. This brief battery was designed to allow clinicians to monitor cognitive change and better inform treatment decisions. It may also serve researchers who want an estimate of global cognitive function without requiring a full neuropsychological battery.

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61. The Effects of Mood on Social Cognition-Interpersonal Perceptions- in Mood Disordered Patients

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Background: A growing body of information suggests that there is a close relationship between negative affects, including depression, hostility and anxiety and the negative interpersonal perception of another person, or in the perception of moods as seen in pictures of faces. Furthermore, the author and others have found that the stimulant drug methylphenidate, and other drugs of abuse can alter such perceptions.

Methods: In the current study, 14 psychiatric patients with mood disorders were administered the Profile of Mood States (POMs), a self-administered mood rating scale. They were then asked to rate their closest significant other (i.e. spouse, best friend, etc.), using the Barrett Lennard Relationship Inventory, a scale originally developed to evaluate positive interpersonal variables in psychotherapists, measuring perceived empathy, regard, unconditionality, and genuineness.

Results: A strong negative relationship was observed between the patients' POMs negative affect subscales (i.e. depression, anger, anxiety) and the Barrett Lennard Relationship Inventory total scale scores. A less dramatic positive relationship was found between POMs positive affect subscales and the Barrett Lennard Relationship total score. Selected Barrett Lennard Relationship Inventory subscales were also related to POMs negative affect scales.

Discussion: We have previously shown a close correlation exists between POMs negative mood rating scale scores and low ratings on the Barrett Lennard Relationship Inventory. In this work a research assistant/interviewer was rated by psychiatric inpatients. The current work in part replicates this earlier work, and expands the relationship to the patient-significant other dyad. Consideration of how mood can effect interpersonal interactions and perceptions (ie. social cognition) may add a significant dimension to our understanding of psychopathology of mood disorders, and offer a further dimension concerning the effects of psychotropic agents.

Disclosure: D. Janowsky: Part 2; QRxPharma.

62. The Impact of Ethanol on Attention and Cognitive Inhibition in Rhesus Macaques

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Background: Cognitive control is required to inhibit prepotent responding and adapt responding to changing circumstances.

Decreases in cognitive control have been hypothesized to contribute to the behavioral disinhibition induced by ethanol, but investigation of specific cognitive domains impacted by ethanol has been relatively limited. It is important to develop a better understanding of the impact of ethanol on specific cognitive domains because the dangerous behavioral effects such as aggression, risk-taking and impulsivity, are thought to be a consequence of decreased cognitive control over behavior. In the present study we evaluate the impact of acute administration of a range of physiologically relevant ethanol doses in rhesus monkeys on the cognitive domains of stimulus response, stimulus discrimination, stimulus reversal performance, error-processing, and attention.

Methods: Rhesus macaques (n=7) received intravenous ethanol (0.2, 0.5, or 1 g/kg) or saline prior to performing touch screen-based tasks for water reward in a sound-attenuating chamber. A stimulus discrimination with reversal task was used to determine cognitive inhibition and error processing and a 9-choice serial reaction time task was used to evaluate attentional function.

Results: At 0.5 g/kg, alcohol selectively disrupted stimulus reversal performance without impact on the stimulus response or stimulus discrimination performance. A trend towards the same effect was also observed at 0.2 g/kg (p=0.059). Low doses (0.2 and 0.5 g/kg) of ethanol also disrupted error processing and increased the number of omissions on the attentional task (0.5 g/kg). Impairment of attentional function was further supported by an increase in the frequency of attentional lapses as determined by response time analysis on this task. The highest dose of ethanol (1 g/kg) impaired discrimination performance and prevented determination of reversal performance.

Discussion: These data demonstrate that distinct cognitive domains, mediated by specific prefrontal cortical brain regions, are differentially sensitive to low doses of ethanol. Furthermore, these data suggest specific mechanisms for the impact of ethanol on decision making, which likely contribute to the poor choice behavior even at low doses of alcohol. Supported by NIAAA and the VA Medical Research Service.

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63. Brain Immunophilins, Aging, and Cognitive Impairment

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Background: Cognitive function is an important aspect of successful aging. The neurobiological factors that underlie successful cognitive aging, however, are poorly delineated. Perturbations in oxidative stress signaling are proposed to be important in age-associated cognitive deficits. We have previously shown that short-term recognition memory impairment in aged mice is associated with decreased expression of the immunophilin FK506 binding protein (FKBP) 51 in frontal cortex and hippocampus. These data suggest that there may be aberrant expression of other immunophilins in the aged brain. Though immunophilins are involved in pleiotropic signaling pathways, imbalances in these molecules may have an important impact on oxidative stress signaling. This is suggested by the role of immunophilins in regulating target of rapamycin (TOR), a key protein kinase whose activation results in an accumulation of damaging reactive oxygen species (ROS). Hypothesis: Our overall hypothesis is that perturbations in immunophilin homeostasis lead to impairment of the neuronal response to oxidative stress and result in a loss of synaptic plasticity and cognitive impairment in aged mice.

Methods: We used single-trial object recognition in young (5-month-old) and aged (24-month-old) male C57BL/6N mice (n=20 each) to assess their non-spatial recognition memory. This test measures an animal's ability to recognize a familiar object and discriminate it from a novel object. In the present study we employed quantitative immunohistochemistry (IHC) to study the expression of the immunophilins FKBP12 and FKBP38 as well as nitrotyrosine,

microtubule-associated protein 2 (MAP-2), and synapsin-IIa in the brains of young versus aged mice. We further correlated levels of these molecules in the aged mouse brain with performance on single-trial object recognition. In our ongoing series of investigations, we are also analyzing immunophilin alterations and their relationship to underlying brain circuitry by employing 1,1'-diiodo-3,3,3',3'-tetramethyl-5,5'-bis(4-methyl-6-propylthio)carbocyanine perchlorate (DiI) staining of brain sections. This neuronal tracer will be used to visualize dendrites within FKBP38 granule clusters and within frontal cortex of aged animals. All experimental protocols are approved by the Institutional Animal Care and Use Committee and have been conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: IHC for nitrotyrosine, as well as FKBP38, MAP-2 and synapsin-IIa showed hippocampal granule clusters in aged mice that were nearly absent in young animals, while no differences were observed in FKBP12. Granule cluster immunoreactivity for none of these molecules correlated significantly with performance on single-trial object recognition, however there was a trend toward increased immunophilin expression in age-impaired mice. We are currently expanding the cognitive performance battery to include tests focused on hippocampal circuitries. In addition, we have optimized a protocol for visualizing dendritic spines that utilizes DiI staining of brain sections.

Discussion: Our finding of FKBP38 hippocampal granule clusters only in the older mice reveals an important alteration in immunophilin signaling in the aged brain. Accompanying granule cluster formations of nitrotyrosine suggest an impaired neuronal response to oxidative stress, while clusters of MAP-2 and synapsin-IIa show associated deficits in the structure of hippocampal circuits. These findings add to our previous immunophilin data that have shown decreased FKBP51 in frontal cortex of aged mice. In contrast to decreased expression of FKBP51, which correlated with deficits in single-trial object recognition, hippocampal clusters of FKBP38 did not correlate with deficits on this task. Taken together, these data suggest an important role for immunophilin dysfunction in aging brain that is distributed across different immunophilin molecular types as well as neuroanatomical regions. Our ongoing studies will examine the co-localization of nitrotyrosine, FKBP38, MAP-2, and synapsin-IIa in the aged hippocampus. We will also investigate the co-localization of the clusters with TOR. The role of TOR in oxidative stress signaling and its regulation by immunophilins suggests that it will co-localize with FKBP38 and nitrotyrosine hippocampal clusters. Collectively, these studies should provide important information on age-related immunophilin perturbations, their association with oxidative stress, and their relationship to underlying dendritic circuitry that is considered to be important for cognition.

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64. Dark-Enhanced Startle as a Biomarker of Anxiety is Increased in Children of Abused Mothers

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Background: In the face of high rates of trauma exposure among low income, urban women and their children, understanding the processes

through which maternal PTSD confers risk to their offspring is of critical public health importance. Our laboratory has recently found that childhood abuse is associated with increased startle reactivity in adulthood. Studies of baseline startle and dark-enhanced startle have indicated that these measures may serve as good predictors of risk for psychopathology in children. The current study uses these non-invasive psychophysiological methods to describe biomarkers of anxiety in children.

Methods: The study sample was recruited from a highly traumatized urban population at Grady Hospital in Atlanta, GA. We recruited mother-child pairs ($n=35$), targeting school-age children. Mothers were assessed for early abuse using the Childhood Trauma Questionnaire, as well as PTSD symptoms using the Posttraumatic Symptom Scale (PSS). Children were assessed for psychological symptoms using the Behavior Assessment System for Children (BASC). We measured baseline startle response and dark-enhanced startle using EMG recordings of the eyeblink muscle in the children.

Results: We found that child anxiety symptoms on the BASC were positively correlated with dark-enhanced startle ($r=0.54$, $p<0.05$), but not baseline startle reactivity. We also found that dark-enhanced startle was five times higher in children whose mothers had high levels of childhood physical abuse, compared to children whose mothers had low levels of physical abuse, $F(1,35)=4.99$, $p<0.05$. This effect remained significant after covarying for maternal PTSD symptoms. In fact, after accounting for the child's sex and age, and maternal PTSD symptoms, in a hierarchical regression analysis, maternal childhood trauma accounted for 16.8% of the variance in the child's dark-enhanced startle response, $F_{\text{change}}(1,29)=6.03$, $p=0.02$.

Discussion: These results suggest that high-risk children have higher dark-enhanced startle than low-risk children, and emphasize the utility of startle as a pervasive biomarker of psychopathology that can easily be measured in children. Investigating such precursors of stress-related psychopathology in children is of crucial importance to progress in this field.

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65. Loosening of Associations Revisited: Social Communication Dysfunction in Schizophrenia

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Background: The study of social dysfunction in schizophrenia has emerged as a major research endeavor over the past decade. Most work has focused on non-verbal and affective aspects of social interaction, with relatively little study of the profound verbal communication deficits that also characterize schizophrenia. Language problems in schizophrenia are most clearly manifest as the disorganized speech produced by a subset of patients with positive thought disorder. In these patients, speech is often characterized by an inappropriate intrusion of semantic associates, i.e. "loosening of associations". What is underappreciated, however, is the frequency with which linguistic deficits can impair communication, even when thought disorder is not clinically detectable. For example, many schizophrenia patients fail to establish links between multiple mentions of the same character or object (e.g. linking 'he', 'John' and 'the man'), resulting in confusing or ambiguous verbal communication. Such failures to establish linguistic reference are stable over time and are also observed in first-degree relatives of patients. Despite these observations, there has been virtually no work examining the neurocognitive underpinnings of such verbal communication failures. In the DSM-IV, 'loosening of associations' is used mainly to describe the phenomenology of clinically manifest positive thought disorder. However, Bleuler originally conceived of it as a cognitive mechanism that might explain multiple aspects of psychotic thought. In this study, we used a direct measure of online brain activity - event-related potentials (ERPs) - to test the

hypothesis that failures to establish linguistic reference in schizophrenia stem from a subclinical loosening of association.

Methods: We measured ERPs as 16 schizophrenia patients and 16 demographically-matched controls read five-sentence scenarios. Sentences 4 introduced a noun which referred back to three possible referents introduced in sentences 1-3. These referents were contextually appropriate, contextually inappropriate but lexico-semantically associated, and contextually inappropriate and lexico-semantically non-associated. To determine whether participants had correctly linked a noun to its appropriate referent, the final sentence reintroduced each referent and participants indicated whether the last two sentences referred to the same entity. Analyses were conducted on the mean amplitudes of ERPs evoked by this referent across 300-400 msec and 400-500 msec, yielding a fine-grained analysis of the time-course of the N400 ERP component, known to index online, word-by-word semantic processing.

Results: Group x Referential Condition interactions were observed in both time windows ($F_s > 3.1$, $p_s > 0.5$, but both were significantly smaller than the N400 in the non-associated condition, all $p_s < .05$). This overdependence on lexico-semantic associates directly impacted behavior (Group x Referential Condition interactions for reaction times, $F(2, 60) = 3.63$, $p < .05$, and accuracy, $F(2, 60) = 3.05$, $p < .07$): patients were more likely than controls to incorrectly link the target noun with contextually-inappropriate, but lexico-semantically associated, referents.

Discussion: These results show that an overdependence on semantic associates can lead to failures in establishing linguistic reference. Although patients activated lexico-semantic associates slower than controls, the sustained activation of these associates ultimately overrode the influence of the discourse context. We suggest that such 'slow and sustained' lexico-semantic activation characterizes not only the disorganized speech produced by a subset of patients, but that it reflects a fundamental neurocognitive abnormality which can explain multiple thought and communication failures in schizophrenia.

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66. Neural Correlates Of Human Aggression In Alcohol-dependent And Control Subjects

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Background: Alcohol-related human aggression is a well-documented, complex and problematic phenomenon with profound public health consequences. Studying this relationship by merging methodologies in brain imaging and laboratory behavioral science will further scientific understanding of this complex phenomenon. Here we apply functional magnetic resonance imaging (fMRI) to examine neural correlates that mediate the relationship between human aggressive behavior and chronic alcohol use.

Methods: This experiment utilizes a laboratory model of human aggressive behavior (the Point Subtraction Aggression Paradigm, or PSAP) adapted for use during fMRI, to study differences in brain networks underlying aggressive behavior among alcohol dependent and matched healthy control participants. BOLD activation is measured during bouts of operationally-defined aggressive behavior (provoked by monetary subtractions) relative to responding on a different option that produces monetary earnings. To date, 8 subjects meeting DSM-IV criteria for lifetime alcohol dependence and 12 healthy controls have completed the protocol. All subjects were drug and alcohol free on testing days. Whole brain random-effects analyses using SPM8 examined group differences in brain regions relevant to

both chronic alcohol use and human aggressive behavior, including regions related to emotion and behavioral control.

Results: Data were analyzed by examining group contrasts between bouts of monetary-earning responding, aggressive responding, and periods of provocation, in which subjects were provoked by having subtracted from their total by a fictitious opponent. SPM8 analysis of the regression of provocation_minus_monetary-response activation on aggressive/monetary response ratio showed one cluster in which the normal control group had significantly (FWE-corrected) greater regression slope than the alcohol dependent group. Areas that showed significantly greater regression slope for the control group included portions of R middle and R superior medial frontal gyrus, R superior temporal gyrus, R caudate, R putamen, and R insula. There were no clusters in which the alcohol group had greater regression slope than the control group for provocation_minus_monetary response activation on aggressive/monetary response ratio. SPM8 analysis of the regression of aggressive_minus_monetary-response activation on aggressive/monetary response ratio showed one cluster in which the alcohol dependent group had significantly (FWE-corrected) greater regression slope than the normal control group. Areas that showed significantly greater regression slope for the alcohol group included portions of L inferior and middle frontal gyrus, L middle cingulate gyrus, L precentral gyrus, L parietal lobule, L angular gyrus, and L middle occipital gyrus. There were no clusters in which the control group had significantly greater regression slope than the alcohol group for the regression of aggressive_minus_monetary-response activation on aggressive/monetary response ratio.

Discussion: Individuals who abuse alcohol are at increased risk for aggressive behavior. Here we submit preliminary evidence, under controlled laboratory/imaging conditions, demonstrating that individuals with past alcohol dependence show higher levels of aggressive responding and differences in BOLD activation in brain regions known to correspond to emotion, behavioral control, and human aggressive behavior.

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67. Neural Mechanisms of Mental State Attribution in Schizophrenia

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Background: Mental state attribution refers to the ability to explain and predict behaviors of oneself and others based on mental states such as belief or thought. Mental state attribution is critical for understanding and predicting others' behaviors, and thus it plays a key role in successful social behavior. Previous behavioral studies have shown that schizophrenia patients have impaired mental state attribution, which is associated with impaired social functioning of patients. However, our knowledge of neural mechanisms associated with mental state attribution in schizophrenia is very limited. This study aimed to identify the neural mechanisms of mental state attribution in schizophrenia using a well-validated functional magnetic resonance imaging (fMRI) paradigm in social neuroscience: the Belief Attribution Task.

Methods: Thirteen schizophrenia patients and 13 healthy control subjects performed the Belief Attribution Task in a 3T Siemens scanner. The Belief Attribution task consisted of a false belief condition and a false photograph condition. For the false belief condition, subjects were asked to read stories about a character's belief and predict his/her behavior based on that belief. In these stories, a character's belief is false in that it differs from the true state of affairs; and the behavior inferred by this false belief differs from the behavior that would be predicted by the true state of affairs. To perform the false belief condition, subjects must be able to attribute the belief of a character despite the belief being false. The false photograph condition was intended to control for the general problem-solving structure of the false belief story. The false photograph story has the same story

structure (e.g., the same complexity of the structure) without the component of belief attribution. We analyzed fMRI data using the FMRIB Software Library (FSL).

Results: For the false photograph condition, both schizophrenia patients and healthy controls showed increased activations in the inferior frontal gyrus, middle temporal gyrus and thalamus. There was no group difference in these regions. For the false belief condition, both groups showed task-related activations in the inferior frontal gyrus, middle temporal gyrus, temporo-parietal junction, and putamen. However, compared to schizophrenia patients, healthy controls showed increased activations in bilateral temporo-parietal junction and left middle temporal gyrus. Further, increased activations in the temporo-parietal junction during the false belief vs. false photograph condition were observed in healthy controls, but not in schizophrenia patients.

Discussion: This study examined the neural correlates of belief attribution in schizophrenia patients using fMRI. For the false photograph condition that requires problem solving abilities similar to the false belief condition, schizophrenia patients showed neural activation patterns similar to those of controls. However, during the false belief condition, healthy controls exhibited increased activations in the temporo-parietal junction and the middle temporal gyrus, relative to schizophrenia patients. The increased activation in the temporo-parietal junction during the belief attribution is consistent with previous studies of healthy individuals. The current findings suggest that reduced activations in the temporo-parietal junction of schizophrenia patients are associated with impaired belief attribution.

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68. Longitudinal Course of Neurocognitive Functioning in Psychotic Disorders

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Background: Neurocognitive dysfunction is a core feature of psychotic illness and may both reflect neurodevelopmental pathology and predict outcomes in patients. Past comparisons of neurocognitive dysfunction across diagnoses have yielded inconsistent findings. Additionally, the course of neurocognitive functioning over time and in relationship to other symptoms is unclear. *Goals and Hypotheses:* We examined neurocognitive functioning in a cross-diagnostic sample of patients with psychotic disorders at baseline and again 6 months later. Based on previous reports, we predicted that 1) all patient groups would exhibit neurocognitive deficits compared to controls, but would not differ from each other, 2) negative symptoms (but not mood, positive, or general symptoms) would be associated with poorer cognitive functioning, 3) neurocognitive deficits would remain stable over the follow-up interval in all patient groups, and 4) neurocognitive change would not correlate with clinical change.

Method: Patients with schizophrenia (SZ; $n = 25$), schizoaffective disorder (SZA; $n = 29$) and bipolar disorder (BD; $n = 31$) and healthy controls ($n = 20$) were recruited from the Schizophrenia and Bipolar Disorder Program at McLean Hospital and administered a clinical and cognitive assessment at baseline and 6 months later. The cognitive battery included measures of executive functioning (Trails B, Stroop Color-Word), verbal and spatial memory (HVLT, BVMT), verbal fluency (Category Fluency) and processing speed (Trails A, Stroop Color); the clinical battery included a structured diagnostic interview (baseline only), YMRS, MADRS, and PANSS.

Results: Baseline and preliminary follow-up data ($n = 43$) are available. At baseline, all patient groups exhibited cognitive deficits relative to healthy controls. Patients with SZ performed worse than patients with SZA and BD on Trails B; patient groups did not differ on any other neurocognitive measure. Linear regressions controlling for demographic variables revealed that cognitive functioning at baseline did not differ by diagnosis. When symptom scores were added to the

model, negative symptoms were predictive of performance on Trails B and Stroop Color-Word; neurocognitive performance was not predicted by any other clinical measure. At follow-up, patients exhibited clinical improvement on most measures; however, all patient groups continued to exhibit significant cognitive deficits compared to controls, and did not differ from each other on any neurocognitive measure. Patients with BD showed significantly greater change at follow-up on Stroop Color-Word than patients with SZ or SZA; neurocognitive change scores did not differ by diagnosis on any other measure. A series of linear regressions revealed that neurocognition at follow-up was predicted by cognitive score at baseline but not diagnosis or state clinical symptoms. Additionally, clinical change did not predict cognitive change on any neurocognitive measure.

Discussion: All patient groups exhibited significant and similar neurocognitive dysfunction that did not remit with clinical improvement. Across diagnoses, negative symptoms were predictive of executive functioning deficits, while positive and mood symptoms were not associated with any neurocognitive domain. Cognitive symptoms do not appear to follow the same premorbid developmental course between patient groups; however, post-onset, patients exhibit similar deficits that are not diagnosis-dependent or secondary to clinical state. Further comparisons of the timing and course of neurocognitive development may inform the examination of etiological factors that differentiate patient groups, and those that are shared. Additionally, given its relationship to global outcomes in patients, cognitive dysfunction is an important treatment target in all patients with psychotic illness.

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69. Renewing and Reinstating Fear Responses in PTSD Patients and Underlying Neurocorrelates

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Background: Post Traumatic Stress Disorder (PTSD) is a chronic and debilitating disorder associated with heightened fear responses. Recent studies have utilized fear conditioning paradigms to investigate the mechanisms underlying increased fear responding in PTSD patients (e.g. Milad et al., 2005; Jovanovic et al., 2009). Using a fear conditioning paradigm in conjunction with fMRI, we aimed to investigate conditions conducive to renewing and reinstating fear responses in PTSD and control individuals, and the neurocircuitry which subserves these effects. Specifically, we investigated the role of a danger context in renewing fear responses, and the propensity for different reinstatement procedures (unpredictable electric shocks vs. threat pictures) to reinstate fear responses to formally extinguished stimuli.

Methods: Twenty eight recently returned OEF/OIF veterans [14 PTSD patients and 14 combat control participants (CC)] were recruited from the Ann Arbor VA and took part in a two day fear conditioning procedure in a 3T fMRI environment. During day 1, participants underwent conditioned fear acquisition (pairing of lights with shock [CS+] or no shock [CS-]) in a “danger context” and subsequent fear extinction (repeated presentation of CS+ in the absence of shock (i.e. CS+E) interspersed with CS-) in a “safety” context. Day 2, subjects were tested for extinction recall, and then representation of the CSs in the “danger” context, followed by two procedures designed to reinstate fear: presentation of unpredictable shocks, and presentation of general-threat and war-specific pictures. Each reinstatement procedure was followed by a test of reinstatement, involving repeated presentation of CS+E and CS-. The order of the reinstatement procedures and their subsequent tests of reinstatement were counterbalanced across participants. This abstract will discuss differential brain activation to the conditioned stimuli as a function of renewal and as a result of the two reinstatement procedures within the two groups.

Results: The renewal of the danger context resulted in increased activation of the vmPFC $[(-9\ 54\ -15)\ 29\ \text{voxels}, Z=3.9, p>.001]$ and temporal lobe $[(-54\ -30\ -12)\ 28\ \text{voxels}, Z=3.15, p=.001]$ in the CC relative to the PTSD group in response to the previously extinguished stimulus (CS + E) relative to fixation. In contrast, PTSD participants had increased overall activation following the reinstatement procedures. Viewing war-specific and general-threat pictures resulted in increased neural activity to the extinguished stimuli in the PTSD group relative to the control group. For the contrast CS + E > CS- in PTSD > CC, patients had increased right amygdala $[(15\ -3\ -12)\ 16\ \text{voxels}, Z=3.14, p=.001]$. Following the reinstatement shock procedure, PTSD patients had significantly greater hippocampus activation to the formally extinguished stimuli, relative to the CC group $[(33\ -15\ -21)\ 25\ \text{voxels}, Z=3.23, p=.001]$.

Discussion: Contextual modulation of brain activity to formally conditioned stimuli differed as a function of PTSD status, with CC participants displaying a particular sensitivity to the danger context as displayed by increased activity pertaining to memory. In contrast, the reinstatement procedures served to greater increase fear signal to previously extinguished stimuli in the PTSD patients relative to control participants. In particular, viewing of general threat and war-specific pictures resulted in increased amygdala activity in PTSD patients relative to combat controls. These data suggest that different conditions can elicit modulation of neural responses to stimuli previously associated with fear, and suggest a dissociation between the conditions salient to PTSD participants and controls. A deficit in contextual processing associated with PTSD may underlie the relative failure of the danger context to modulate neuronal signal to stimuli in this population, with PTSD participants failing to differentiate safety from danger contexts. In contrast, PTSD patients may be particularly sensitive to negative cues, especially those connected to the original trauma, to reinstate fear responding. These data help to understand the conditions and neurocircuitry which contribute to persistent and recurrent fear responses in PTSD patients.

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70. This poster has been cancelled.

71. Role of Medial Prefrontal and Parietal Cortex in Self-Enhancement Bias

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Background: Much research has been done on positive self-evaluation and its relationship to mental health. However, little is known about its neural underpinnings. One study (Kwan et al., 2007) has demonstrated that transcranial magnetic stimulation (TMS) to medial prefrontal cortex (MPFC) and midline parietal cortex can reduce self-enhancement bias in a personality trait rating task. Here, we extend those findings by both widening the temporal range of stimulation and by examining MPFC and two other sites activated in PET imaging by the task, right and left lateral parietal cortex.

Methods: Twenty healthy volunteers rated both themselves and their best friend on a set of visually presented adjectives, evenly divided between desirable and undesirable traits. A second group of healthy volunteers performed the same task, with single pulse TMS applied in each trial. As individual adjectives appeared on a monitor, TMS was applied at five different times relative to stimulus onset (SOA: stimulus onset asynchrony) ranging from 0-480 ms. Subjects made yes/no decisions with hand-held buttons about whether or not the adjectives

described themselves or, at other times, their best friend. This procedure was repeated with the TMS coil positioned over three locations (MPFC and left and right lateral parietal cortex) over a single session. TMS sites were found with Brainsight, a computerized frameless stereotaxy system (Rogue Research, Montreal, Canada) using individual MRIs.

Results: In the first group of 20 subjects not receiving TMS, there was a significant 7% bias in attributing desirable adjectives to one's self as opposed to the best friend ($p < 0.01$). There was no difference in attribution of undesirable traits between self and best friend. In the second group receiving TMS, the bias remained positive (i.e., self-enhancement) at the early SOAs at all three sites. At 160 ms SOA, the bias was nullified by TMS at the right parietal site, and became negative (favoring best friend) with TMS to MPFC. There was a corresponding drop at 240 ms SOA for the left parietal site. The bias showed a recovery towards positive values at 480 ms SOA. An omnibus ANOVA revealed significant site and SOA effects ($p < 0.05$).

Discussion: This study, in conjunction with Kwan et al. (2007), demonstrates the effectiveness of TMS in manipulating the self-enhancement bias in a site- and latency-specific manner. TMS may thus be of use in investigating areas of mental illness in which self-evaluation is abnormal, potentially as a diagnostic tool. Also of considerable interest is that the prefrontal and parietal sites affected by TMS and shown to be activated by a PET study of the adjective self/other task, are the primary nodes of the default network discussed in the brain imaging literature. This study extends our previous findings using image guided-TMS (Lou et al., 2004; Lubner et al., submitted) that the default network is integrally involved with self-specific processing.

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72. Emotional Modulation of Response Inhibition in Stable Patients with Bipolar I Disorder: A Comparison with Healthy and Schizophrenia Subjects

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Background: Bipolar disorder (BD) has been associated with impairment in affective processing during depressive and manic states; however, there are limited data on affective processing in BD during periods of mood stability. We examined the pattern of affective processing in stable BDI patients and compared their profile to healthy controls (HC). Additionally, to assess the diagnostic specificity of the patterns of performance, we compared BD patients' performance versus the performance of patients diagnosed with schizophrenia (SZ).

Methods: A total of 336 subjects (mean age = 40.4 ± 11.9 years; 34% female; 34% Caucasian; mean premorbid IQ = 96.4 ± 11.5) were administered an Affective Go/No-Go test to evaluate inhibitory response to negatively-valenced, positively-valenced, and neutral stimuli. Subjects included 59 stable patients with bipolar I disorder, 133 remitted patients with schizophrenia, and 144 healthy controls. Symptomatic stability was defined as Hamilton Depression ratings and Clinician Administered Mania ratings of ≤ 12 at the time of assessment. The Affective Go/No-Go test was comprised of three conditions: discrimination of positively valenced words (e.g. sunshine, candy), discrimination of negatively valenced words (e.g. gun, death), and discrimination of animacy in words of neutral valence (e.g. dog, house). Accuracy (d') and response bias (β) served as dependent variables in a series of multivariate analyses of covariance (MANCOVAs) to test for diagnostic group differences. In addition, two backward likelihood ratio regression analyses (predicting BD vs HC status and BD vs SZ status, respectively) were conducted to assess the specificity of the results.

Results: Analyses revealed that, as expected, healthy individuals tended to respond more accurately when presented with affective information than when neutral stimuli were presented. The response profile of the stable bipolar patients differed significantly from the healthy controls, such that they were more willing to respond to the negatively valenced target stimuli, indicative of a response bias toward negative information ($p < .01$). In addition, a deficit was evident in their response to positive stimuli, as their ability to discriminate and respond accurately to positively valenced information was impaired during the *positive* condition ($p < .05$). In contrast to the pattern noted in BD, patients diagnosed with SZ performed poorly on all aspects of the task relative to healthy subjects and were less accurate across all conditions regardless of affective valence ($p < .01$). Patients with SZ evidenced a reverse bias for positive information such that they were significantly *less* likely to respond to positive words than healthy controls ($p < .05$) despite comparable response bias on neutral and negative conditions. Regression results indicated that differential affective processing profiles on the Affective Go/No-go task were able to reliably predict the group status of the BD patients in comparison with healthy subjects [area under the curve = .78 ($p < .001$), confidence interval of .70-.86; optimal sensitivity = .69, specificity = .80] and versus schizophrenia patients [area under the curve = .78 ($p < .001$), confidence interval of .71-.86; optimal sensitivity = .71, specificity = .68].

Discussion: The affective processing impairment evident in BD patients is a feature of the disorder that is present even during stable periods. In the search to identify cognitive deficits that are specific to BD, prior studies comparing BD with SZ have highlighted clear quantitative but not consistent qualitative differences. Our data suggest that a response bias associated with *negative* stimuli may be a critical and relatively specific feature of BD.

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73. Neural Indices of Abnormal Response Monitoring in Obsessive-Compulsive Disorder

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Background: Adaptive, flexible behavior depends on intact 'response monitoring', which involves detecting errors, evaluating what went wrong, and adjusting behavior to optimize outcomes. Response monitoring relies on the anterior cingulate cortex (ACC) and there is growing evidence that abnormal ACC function in obsessive-compulsive disorder (OCD) contributes to core symptoms. In OCD, the ACC shows increased activation during symptom provocation, cingulotomy relieves obsessions and compulsions, and the therapeutic efficacy of selective serotonin reuptake inhibitors is paralleled by a normalization of ACC activity. During error commission, individuals with OCD show exaggerated responses in the ACC, both increased functional MRI (fMRI) activation and increased amplitude of the error-related negativity amplitude (ERN: an electrophysiological response to errors that is generated in the ACC), which are associated with the severity of obsessions and compulsions. These exaggerated and inappropriate error signals have been proposed to contribute to the pervasive sense of incompleteness and self-doubt that characterizes OCD, and to trigger behavioral repetition in an effort to reduce error signals. Our preliminary findings support this theory.

Methods: Ten adult outpatients with OCD and 14 demographically-matched healthy controls performed an antisaccade paradigm during fMRI in a 3T Siemens Tim Trio scanner. Antisaccades require inhibition of the prepotent response of looking toward a suddenly appearing visual target and the substitution of a gaze in the opposite direction. Eye position was monitored using the ISCAN fMRI Remote

Eye Tracking Laboratory and trials were scored for accuracy and latency. Finite impulse response (FIR) models, as implemented in the FreeSurfer Functional Analysis Stream, were used to provide unbiased estimates of the event-related hemodynamic responses at each time point. Groups were compared on error-related activation in the dorsal ACC (dACC), which is based on the contrast of error vs. correct trials at 6 s.

Results: The groups did not differ significantly in antisaccade error rate or in the latency of correct trials. We replicated the well-established finding of greater activation in the dACC for error compared to correct responses. In OCD, consistent with our hypotheses, the hemodynamic response to errors was exaggerated in bilateral dACC and was significantly greater and also more prolonged than that of healthy controls. Moreover, in the OCD group, greater error-related activation in dACC predicted increased symptom severity (YBOCS total score) in bilateral ACC and posterior cingulate cortex.

Discussion: The stronger and more prolonged HDR to errors in OCD is consistent with the theory that OCD is characterized by exaggerated and inappropriate error signals, and that the focus of this abnormality is the ACC. The correlation between error-related activation and symptom severity suggests that exaggerated and inappropriate error signals contribute to symptoms, perhaps by prompting excessive evaluation and repetition of responses. We are now examining the electrophysiologic and structural correlates of abnormal response monitoring in OCD. This line of investigation holds great promise for illuminating the nature and neural basis of a fundamental cognitive abnormality that contributes to the core symptoms of OCD.

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74. Phasic Dopamine Transmission Scales with Reward Preference and Integrates Reward History in Rats Performing a Dual Reward Discrimination Task

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Background: Deficits in the capacity to formulate, maintain and update predictive representations of value permeate multiple neuropsychiatric disorders. Subjective reward value informs reward preference and influences consequent choice behavior when making decisions. The subjective value ascribed to a reward predicting stimulus is flexible and changes in a manner dependent on multiple factors including physiological needs, contextual cues and the presence of contrasting rewards. For instance, if a predictive cue is preceded by some reward, then the value of that cue could be modified in some way relative to value of the antecedent reward. In one case, the outcome's value could generalize to the subsequent predictive cue, thereby increasing or decreasing the cue's value in a manner concurrent to its own (induction effect). Alternatively, the value of the predictive cue could be inversely dependent on the value of a previously available reward, thereby shifting the cue's value in a direction inverse to the value of the prior reward outcome (contrast effect). Phasic dopamine (DA) transmission in the nucleus accumbens core (NAC) is thought to mediate the processing of reward value. It is therefore reasonable to hypothesize that phasic DA transmission elicited by reward predicting cues adjusts in a manner that depends not only on the value of the predictive cue, but also on the value of the antecedent reward. The purpose of the following experiment is to delineate how the magnitudes transient DA signals (elicited by discriminative, reward predicting cues) vary as a function of prior reward outcomes.

Methods: Rats were trained to perform a Dual Reward Discrimination (DRD) task that requires the detection and discrimination of 2 spatially-distinct cues (panel lights). Illumination of the left cue predicts food (45 mg pellet) whereas that the right cue predicts water (75 µl). Testing consists of 60 forced trials (30 food, 30 water)

presented in random order with 30 choice trials. During forced trials a single cue is illuminated and after 2 seconds, both levers extend into the operant chamber. Rats are required to operate the lever beneath the illuminated cue in order to receive the reward associated with that cue. Incorrect responses (i.e. operation of the non-illuminated lever) yield no reward. During choice trials, both cue lights are simultaneously lit and rats can choose to operate either lever in order to receive their preferred reward; choice trials are then used to calculate each rat's reward preference (preferred vs. less-preferred). Prior to training and surgical recovery, rats received chronic implantation of carbon-fiber microelectrodes in the NAC to conduct fast-scan cyclic voltametry for the assessment of DA transmission in real-time during operant performance.

Results: Initial analysis of DA transients examined trials categorized on the basis of reward preference and compared peak DA signals elicited by preferred cues, less preferred cues, and no-reward cues (e.g. misses). As expected, the transient DA signals elicited by preferred cues were of greater magnitude than those elicited by less-preferred cues and the smallest DA transients were evoked during zero-reward trials. Trials were then sorted on the basis of whether their preceding reward outcomes were preferred or less-preferred. Preliminary analyses of these data indicate that phasic DA transmission elicited by discriminative cues is subject to induction-like effects. Specifically, the peak DA signals observed during both preferred and non-preferred cues become augmented when those cues are preceded by the rats' favorite reward outcome. Moreover, the magnitude of phasic DA elicited by less-preferred cues becomes attenuated when the prior reward outcome was less-preferred. Interestingly, trials that were preceded by zero-reward outcomes evoked the greatest peak DA responses, indicating that contrast-like effects become apparent only when the antecedent outcome is of no value whatsoever. In this case, the zero-value outcome of the previous trial results in an increased lag between reward events increasing the salience of the next detected cue attribution of subjective value to the next correctly detected cue and increasing phasic DA transmission in synchrony.

Discussion: Collectively, these data demonstrate that in the NAC, phasic dopamine events elicited by discriminative, reward-predicting cues reflect not only preference, but also integrate information regarding reward history. A more detailed history-integration analysis will determine the temporal degree to which prior reward outcomes affect cue-evoked DA transmission.

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75. Multi-Site fMRI Study of Cognitive Control-related Brain Activation in Early Schizophrenia and Clinical-High-Risk Youth

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Background: Impairment in cognitive control has been consistently demonstrated in individuals with schizophrenia at all stages of the illness, and is associated with decreased activation of a prefrontally-mediated cognitive control network. However, it is unclear how such functional deficits manifest prior to illness onset. A modified AX-CPT was used as part of a cross-site fMRI study of clinical-high-risk for psychosis (CHR), early psychosis (EP) and healthy control (HC) participants. We hypothesized that EPs and CHRs would show impaired cognitive control along with reductions in prefrontal activation relative to HCs.

Methods: EP (n = 27), CHR (n = 26) and HC (n = 36) participants from the UC Davis Imaging Research Center and UCSF Brain Imaging and EEG Laboratory were identified using the Structured Interview for Prodromal Syndromes (SIPS) and Structured Clinical Interview for DSM-IV (SCID-I/P). Participants performed the modified AX-CPT, a computerized measure of cognitive control, during fMRI scanning.

Results: Behavioral and imaging findings were consistent across sites. EP participants demonstrated a specific pattern of performance indicative of impaired cognitive control on the AXCP and reduced cognitive control-related activation of the prefrontal cortex when compared to controls. Although their behavioral performance did not differ significantly from HCs, CHR individuals demonstrated a pattern of brain activation that was similar to EPs, with reduced activation in prefrontal and other areas of the cognitive control network.

Discussion: Convergence of findings across sites supports the feasibility of collaborative multisite fMRI studies, which are necessary to generate the large data sets needed to understand the cognitive and neural mechanisms underlying risk for psychosis and the transition from risk state to illness. Results provide robust evidence that prefrontal cognitive control mechanisms are disrupted in the early stages of psychosis and may serve as markers of risk. The greater sensitivity of fMRI versus behavioral measures to CHR status also points to the value of obtaining imaging data in high risk studies. Additional analyses in an enlarged sample will examine the relationships between behavior, brain activity, and clinical and functional outcome in CHR and EP individuals.

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76. Abnormalities in Social Cognition Related to Gaze Processing Abnormal in Schizophrenia For Conditions With Higher Processing Demands

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Background: Recent years have seen a rapid increase in the interest in social cognition from fewer than 10 papers in 1997 to nearly 80 in 2007. Social cognition has been defined as human ability to predict and understand the intentions and emotional states of others. Its role in human interactions, its influence on cognition and its importance for a full understanding of schizophrenia symptoms has become clearly recognized (e.g., Barch, 2008). Social cognition has an important role in everyday functioning of schizophrenia patients (Couture 2006; Addington 2006) and is related to functional outcomes (Penn 2008). It has been demonstrated in several studies that schizophrenia patients have abnormalities in structural face processing (Onitsuka et al., 2006). It has been further proposed that abnormalities in basic-level visual processes contribute significantly to abnormalities in higher-order cognitive processes including social cognition (Javitt et al., 2009; Turetsky et al., 2007). We have used event related potential (ERP) methodology capable of tracking neural events in real time as well as behavioral data to examine the processing of faces and gaze shifts in schizophrenia and normal control individuals. Gaze shifts in both humans and primates are a powerful source of social information related to group dynamics and a majority of emotional and social 'meaning' of a face is determined based on eyes. We assumed that N170, an ERP related to face processing will be abnormal based on previous studies. We also hypothesized that if ERPs in all conditions related to gaze processing are abnormal, this would suggest that social cognition related visual abnormalities can be entirely explained by lower level abnormalities in the structural face processing. However, if the gaze processing related abnormalities were present only in conditions imposing higher processing demands, this would argue against a simple processing stream model, and suggest instead an interaction between sensory-based and higher cognition based abnormalities.

Methods: We have obtained data in 12 normal control and 12 chronic schizophrenia individuals (all male) matched for age, parental SES, IQ, and handedness (all right handed). Stimuli, procedure, and approach

to ERP analyses were identical to Carrick et al. (2007) who demonstrated the ERP correlates of gaze processing. There were 180 displays of three faces (60 per condition) in 3 conditions: 1. group condition: all faces look in the same direction; 2. mutual condition: two faces 'exchange gaze' while the third one 'looks away'; 3. avoid gaze condition: the central face is looking up 'avoiding' the gaze of others. The illusion of 'changing gaze direction' was created by showing two panels one after another - the second one depicted the same faces with eye gaze shifted as described above. The task was to identify the type of gaze by pressing one of the three buttons.

Results: The fewest number of errors were committed in the mutual gaze (easiest) ($p < 0.039$) and most errors in the avoid condition ($p < 0.05$) with no group differences. The N170 to all face configurations was reduced in SZ ($p < 0.01$). P350 and P500, identified in the Carrick study as unique to gaze changes were larger in SZ in the group gaze and avoid gaze conditions ($p < 0.05$) with latencies delayed in the avoid condition ($p < 0.005$).

Discussion: These ERP data suggest that while processes related to structural face processing, indexed by the N170, are abnormal, the social aspects of face processing indexed by P350 and P500 are abnormal only in the conditions placing greater demands on spatial working memory (group and avoiding gaze conditions) and speak against the hypothesis of abnormalities in structural face processing as a sole explanation for impairments in socially appropriate interpretations of human gaze. While structural faces processing abnormalities are undeniable, the level of impairment in social interpretation of faces depends on the complexity of the processing demands.

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77. Differential Impact of Risperidone and Divalproex in Modulating Negative and Positive Emotions During an Affective Working Memory Task in Pediatric Mania

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Background: Patients with pediatric bipolar disorder (PBD) have affect regulation and working memory deficits. Therefore, we hypothesized that the interface of the corresponding affective and working memory circuits is impaired in PBD. We also predicted that the treatment of PBD with second generation antipsychotic and a traditional mood stabilizer (risperidone and divalproex respectively) would result in different mechanisms of action in reversing the brain function.

Methods: This was a six-week double blind randomized trial of risperidone ($n = 10$, 12.4 ± 1.6 years) plus placebo vs. divalproex plus placebo ($n = 11$, 13.1 ± 2.6 years) for manic and mixed episodes of bipolar disorder. The fMRI outcomes were measured using a block design affective N-back task with blocks of angry, happy and neutral faces. Healthy controls (HC) were scanned pre and post trial ($n = 15$, 14.5 ± 2.8 years). Our affective N-back fMRI paradigm is a block design task that assessed the ability to recall an emotional face stimuli that was presented on previous trials. For functional imaging data, FIASCO and AFNI software were used. The primary analysis for this study was a whole-brain voxel-wise 3 x 2 ANOVA.

Results: Divalproex group had slower reaction time and reduced accuracy on follow-up relative to risperidone group or HC. For the angry vs neutral face condition at follow-up relative to baseline, the risperidone group relative to HC showed increased activation in right ventrolateral prefrontal cortex (VLPFC), perigenual anterior cingulate cortex (ACC), posterior cingulate cortex, left subgenual and dorsal ACC, bilateral medial PFC, ventral striatum, putamen, and bilateral middle temporal gyrus. For the angry vs neutral face condition at follow-up relative to baseline, the divalproex group relative to HC showed increased activation in bilateral insula, medial PFC, dorsal caudate and putamen, nucleus accumbens, middle temporal gyrus, and

posterior cingulate gyrus, and left VLPFC and subgenual ACC. On direct comparison between patient groups in angry vs. neutral face condition, left VLPFC and middle temporal gyrus showed greater activation in divalproex group, while left ventral striatum showed greater activation in risperidone group. Additionally, with regard to the divalproex group, there was a significant positive correlation between improvement in Young mania rating scale (YMRS) scores and change in activation in left VLPFC and a non-significant trend in the right VLPFC. The happy face condition was, overall, a less emotionally challenging stimulus for our patients and did not elicit notable findings.

Discussion: With pharmacotherapy, fronto-striatal and fronto-temporal regions are engaged in modulating negative emotion generated by angry faces while accomplishing the working memory task in pediatric mania. Divalproex predominantly engaged the fronto-temporal circuitry both relative to risperidone group and HC. The antiepileptic agent is known to engage the medial temporal lobe (Rinaldi et al, 2008), besides the fact that working memory task recruits the fronto-temporal circuit (Paskavitz et al., 2010). Given that risperidone is a medication known to decrease irritability and modulate anger, the risperidone group predominantly engaged ventral striatum relative to both the divalproex group and HC. This study is taking us a step further in decoding the operational change in neural circuitry function with specific pharmacotherapeutic agents while performing cognitive tasks that are close to real life function, such as engaging in working memory while looking at emotional face expressions.

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78. Alexithymia in Borderline Personality Disorder: Clinical and Behavioral Measures

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Background: Borderline Personality Disorder (BPD) patients show exaggerated physiological responses to emotional pictures along with blunted subjective responses to those pictures. This “disconnect” resembles the construct of alexithymia, which has been shown to be inversely related to measures of empathy. We aimed to explore the association between alexithymia, empathy, and subjective responses to emotional pictures in patients with BPD. We hypothesized that: 1) BPD patients would have higher levels of alexithymia than healthy controls (HC) and lower empathic capacity; 2) BPD patients would be blunted compared to HCs in their subjective rating of both positive and negative emotional pictures; 3) This blunting would be most pronounced in BPD ratings of their own emotional experiences.

Methods: Subjects: 37 HCs with no history of Axis I or II diagnoses and 40 BPD patients, aged 18-65 years. Exclusion criteria included history of serious head injury or neurological disorder; alcohol and/or drug abuse/dependence within prior 6 months; history of a psychotic disorder; current major depressive episode, and psychiatric medication use within six medication half-lives. All subjects completed self-report measures (Toronto Alexithymia Scale, TAS-20; Interpersonal Reactivity Index, IRI) and a subset (29HCs and 30BPDs) viewed the Social Affective Response Task. This task involves viewing positive, neutral and negative emotional pictures with interpersonal content on two separate occasions: 1) First administration: participants were instructed to rate what they believed the people in each picture were feeling (the “Other” condition) on a scale from 1 to 9 (1 = most pleasure, 9 = most pain); 2) Second administration: participants were instructed to imagine themselves in the situation that they were viewing and rate what they would feel (the “Self” condition) on the same scale from 1 to 9 (1 = most pleasure, 9 = most pain).

Results: BPD subjects (TAS total scores mean [M]) = 52.0, SD = 12.6) had significantly higher rates of alexithymia than HCs (M = 34.8, SD = 8.1) ($F = 50.0$, $p < 0.001$). BPD patients had significantly higher alexithymia in the identifying feelings ($F = 73.7$, $p < 0.001$) and describing feelings ($F = 17.3$, $p < 0.001$) subscales, but there were no group differences in externalizing thinking. On the IRI, BPD patients did not differ in empathic concern, fantasy and perspective taking from HCs, but they did score higher (M = 19.5, SD = 4.8) than HCs (M = 13.9, SD = 4.5) on the personal distress subscale ($F = 20.5$, $p < 0.001$). After controlling for Reaction Time, The Diagnosis (HC vs BPD)*Condition (Self vs Other) interaction was significant for the Negative ($F = 7.25$, $p = 0.0102$) and Neutral ($F = 7.52$, $p = 0.009$) pictures, but not for Positive pictures. For Negative pictures, post-hoc tests revealed that BPD subjects’ ratings were less negative in the Self condition relative to HC subjects ($p < 0.05$), whereas ratings were non-significantly different between HCs and BPDs in the neutral and positive valences. There were no significant differences between the groups in the Other condition. This finding did not change by adding Mood State to the model.

Discussion: Our data show the following principal findings: 1) individuals with BPD scored significantly higher in alexithymia than HCs. Unlike in previous reports, however, BPDs did not differ in empathic concern from HCs. The only IRI subscale in which BPD patients differed from HCs was personal distress; 2) and 3) BPD patients showed blunted subjective ratings of emotional pictures compared to HCs only for Negative pictures in the Self condition (ratings of imagined pain for oneself). We confirm higher alexithymia in BPDs compared to HCs in a larger sample than has previously been reported. The blunted response to negative experiences for themselves more than for others is interesting, since BPD patients report themselves to be in constant intrapsychic pain, unrelated to the real significance of external events. We hypothesize that this may be because the pain in imagined interpersonal situations, such as being hit or arrested or other tangible stressors (as pictured in the Social Affective Response Task), pales in comparison with the self-generated pain of their intrapsychic state. In other words, borderline patients have raised the threshold for experiencing pain relative to their own level of intrapsychic pain, and this may bias their ability to imagine their own emotional state in certain interpersonal situations. This is consistent with data of elevated pain thresholds in BPD.

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79. Amygdala-Frontal Circuits in Social Anxiety Disorder: fMRI During Threat Perception and At Rest

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Background: Dysregulated fear is a core process in the etiology and maintenance of anxiety disorders. Convergent evidence implicates frontal-amygdala circuits as central to fear responding and regulation. Although a growing number of functional neuroimaging studies have consistently demonstrated exaggerated amygdala reactivity to threat in patients with social anxiety disorder, few have directly examined amygdala-frontal interactions both when processing threat signals and at rest in the same subjects.

Methods: Using functional magnetic resonance imaging (fMRI), we probed amygdala reactivity to social signals of threat (fearful faces) and non-threat (happy faces) using a well-validated emotional faces matching task in large cohorts of unmedicated subjects with social anxiety disorder - generalized subtype (gSAD, total n = 50) and healthy controls (HC, total n = 25). A large subset of these subjects also underwent ‘resting state’ fMRI scans in the absence of any overt cognitive or emotional task. Here we examined amygdala-frontal interactions using two complementary approaches (SPM5, random

effects): 1) psychophysiological interaction analysis to examine task dependent (threat vs. non-threat) patterns of amygdala-frontal connectivity; and 2) task-independent amygdala-frontal functional connectivity during resting state. Subject-specific connectivity maps were entered into second-level analyses to examine patterns of connectivity within- and between-groups.

Results: Preliminary analyses have yielded 3 main findings: 1) Large-scale, robust and reproducible task-dependent and task-independent connectivity patterns were observed between the amygdala and primary and higher-order sensory cortices in both gSAD and HC groups; 2) In response to threatening faces, gSAD subjects exhibited greater amygdala-ventral frontal connectivity (medial and lateral orbitofrontal cortex) and less amygdala-dorsal frontal connectivity (dorsal medial prefrontal and dorsal anterior cingulate cortex) than HC subjects; and 3) At rest, gSAD subjects also exhibited alterations in amygdala-frontal connectivity but that differed from those observed during task. A number of additional analyses are underway: 1) interactions between task and rest networks; 2) delineation of networks based on basolateral and centromedial amygdala subregions; 3) links between indices of connectivity and symptom severity.

Discussion: Using different fMRI tasks and connectivity analyses, we dissociated the patterns of amygdala-frontal interactions in gSAD patients and healthy controls. Although both groups shared reproducible, robust patterns of connectivity, we find that gSAD patients exhibited altered patterns of amygdala-frontal coupling specifically when processing social signals of threat (e.g., not observable when processing non-threatening information or at rest). These findings extend and clarify existing evidence of amygdala dysfunction and threat-biased processing in gSAD.

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80. Impaired Striatum-Dependent Implicit Learning in Obsessive-Compulsive Disorder

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Background: Obsessive-compulsive disorder is characterized by intrusive, perseverative, anxiety-provoking patterns of thought and ritualized behaviors performed to reduce or contain that anxiety. Convergent anatomical and functional imaging studies have identified the striatum, along with orbitofrontal and anterior cingulate cortices, as being pathologically hyperactive in OCD. The striatum is the input nucleus of the basal ganglia and has a key role in the formation of automated patterns of behavior and thought - habits. Indeed, the inflexible nature of obsessions resembles a habitual pattern of thought carried to a pathological extreme. It has therefore been hypothesized that patients with OCD may have deficits in normal striatum-dependent habit-like learning. Indeed, a few studies suggest deficits in such learning tasks, or abnormal recruitment of the striatum during performance of such tasks. We tested whether patients with OCD exhibit impaired performance on a probabilistic classification task (the Weather Prediction Task) thought to depend on normal striatal function.

Methods: 30 adult patients with OCD (13 med-free) and 20 controls were tested on the Weather Prediction Task. The primary outcome was change from baseline (defined as performance in trials 1-10) in classification accuracy over the course of training. Post-tests assayed subjects' explicit learning during the task. Data were analyzed by RM-ANOVA, covarying for age, sex, and IQ as assayed by the WISC-IV.

Results: Patients with OCD were significantly impaired in learning the Weather Prediction Task across learning trials. This effect was present in both medicated and medication-free patients. Subjects with OCD and controls showed similar explicit learning during the task, as assayed by a post-test.

Discussion: These results provide evidence for a functional deficit in striatum-dependent habit-like learning in obsessive-compulsive disorder. Pathology in habit learning circuits may contribute to the inflexible cognitive patterns underlying the symptomatology of this disorder.

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81. Knowing to Remember: Dual Process Signal Detection (DPSD) Analysis of Recollection and Familiarity in Schizophrenia

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Background: Recognition memory can be supported by either assessments of the familiarity (F) of studied items, or by recollection (R) of contextual details associated with the study event. For example, when you see a person on the street you can have a sense that you met them before but be unable to retrieve the context or details of your meeting (a familiarity based memory), or you can vividly retrieve where you met them, who they are, and what you last talked about (a recollection based memory). It has yet to be established whether patients with schizophrenia have more prominent familiarity or recollection deficits. Answering this question may inform pathophysiology and treatment development as these two processes can be anatomically dissociated within the prefrontal and medial temporal lobe using fMRI.

Methods: This quantitative review identified 17 studies that used remember/know (R/K) or source retrieval paradigms to address this question. A dual process signal detection (DPSD) analysis of reported study results was used to generate quantitative F and R parameter estimates. We also performed DPSD analysis of our own preliminary data from three memory studies.

Results: Although many previous R/K studies concluded that patients had primary recollection deficits, the DPSD analysis revealed deficits in both R and F. Consistent R and F deficits were also observed in our own data.

Discussion: These results suggest that memory impairments in individuals with schizophrenia are secondary to difficulties retrieving contextual details of the encoding event and to problems using a subjective sense of familiarity to guide signal detection processes. Further, our findings imply that these memory deficits in patients with schizophrenia are not solely due to dysfunction in the medial temporal lobe (hippocampus and perirhinal cortex), and could also be explained by dysfunction in prefrontal or other cortical brain regions. Combined fMRI and DPSD modeling is clearly warranted to better establish these functional and anatomical correlates.

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82. Metformin for Obesity and Metabolic Abnormalities in Schizophrenia

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Background: Many antipsychotic medications have been associated with weight gain and metabolic abnormalities. Patients taking antipsychotics have a high incidence of obesity, dyslipidemia and diabetes mellitus. Treatments for these conditions are usually complex, requiring a combination of behavioral and pharmacological interventions. For obesity, in particular, several FDA-approved treatments are relatively contraindicated in patients with psychotic disorders, leaving

few viable options. Interestingly, metformin - a safe and well-tolerated biguanide antihyperglycemic drug - is associated with weight loss in patients with type II diabetes mellitus. A number of smaller studies have also found that metformin augmentation can help produce modest weight loss in children and young adults with new-onset schizophrenia who have developed antipsychotic weight gain. The potential to achieve weight loss with metformin in the more common scenario of an obese individual with chronic schizophrenia, regardless of antipsychotic agent, is not well understood. The current study tested the hypothesis that metformin is effective for weight loss in patients with chronic schizophrenia or schizoaffective disorder who are currently overweight and taking one or two FDA-approved antipsychotic medications at stable doses. Effects on lipids and glucose metabolism were also assessed.

Methods: In a 16-week, double-blind, randomized trial, 148 outpatients with schizophrenia or schizoaffective disorder with a body mass index (BMI) $>27\text{ kg/m}^2$ received metformin, titrated to 2000 mg/d, as tolerated, or placebo. All patients also received a weekly behavioral intervention focused on improving diet and exercise habits.

Results: Metformin was associated with significantly greater weight loss than placebo (2.9 kg [95% CI, 1.9-3.9] vs 1.1 kg [95% CI, 0.0-2.1], $p = 0.0092$). Metformin showed a linear group by time interaction on body weight over 16 weeks. Among secondary outcomes, an advantage for metformin was seen for triglycerides. In secondary analyses, metformin appeared to show differential efficacy in patients taking higher (e.g. clozapine, olanzapine, quetiapine) versus lower (e.g. aripiprazole, ziprasidone, haloperidol) metabolic risk antipsychotics on weight, total cholesterol and triglycerides. Metformin produced transient gastrointestinal symptoms (e.g. nausea, diarrhea and bloating) in 10-20% of patients but was otherwise well tolerated.

Discussion: Adjunctive metformin for 16 weeks was safe and effective in reducing weight and triglyceride levels among overweight patients with chronic schizophrenia and schizoaffective disorder who were taking any stable regimen of one or two antipsychotics. Metformin may be more effective in patients taking antipsychotics with a higher metabolic-risk profile. Future studies need to address the effects of continued treatment with metformin on weight and other cardiometabolic risk factors and whether any such benefits can be maintained.

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83. Ziprasidone and the QT Interval: A Comprehensive Review

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Background: The Pfizer clinical development program for ziprasidone has included detailed investigations of QT effects, beginning with several phase 1 pharmacokinetic studies. Subsequently, the phase 2 and 3 short-term, double-blind, placebo-controlled trials of ziprasidone (80-160 mg/d) confirmed a small mean increase in the corrected QT interval (QTc). QTc effects continued to be carefully studied in all phase 4 Pfizer-sponsored ziprasidone clinical trials, and the potential "real world" effects of QTc prolongation were evaluated through postmarketing safety surveillance and a large simple trial (Ziprasidone Observational Study of Cardiac Outcomes [ZODIAC]). A comprehensive review of this extensive database may provide practitioners and researchers an essential reference on this critical safety issue.

Methods: Results of phase 1 pharmacokinetic analyses are summarized. All Pfizer-sponsored, randomized, controlled clinical trials of ziprasidone were included in an analysis of pooled data concerning the QTc. Here we report all data using the Fridericia correction (QTcF) for heart rate. Baseline to end point change (mean, SD, median, and range), QTc prolongation (categorical increase of ≥ 30 , 60, and 75 msec), and peak measured QTc (categorical threshold of ≥ 450 , 480, and 500 msec) were evaluated. In addition, maximal QTc prolongation was plotted against

baseline QTc measurements. All postmarketing safety reports were analyzed using MedDRA query criteria for cardiac events and the results summarized. We also include the primary outcome measure of the ZODIAC trial, in which patients were followed for 1 year by unblinded investigators providing usual care and the primary outcome was nonsuicide mortality in the year after initiation of assigned treatment.

Results: Pharmacokinetic analyses in adults receiving oral ziprasidone demonstrated dose-dependent increases in QTc over the range of 40 to 160 mg/d, with a limited increase at a higher dose (19.5 msec mean increase at 160 mg/d vs 22.5 msec at 320 mg/d), with no subject reaching a QTc ≥ 450 msec. Pharmacokinetic analyses using intramuscular (IM) ziprasidone yielded similar results, with no subject reaching a QTc ≥ 480 msec. In total, 3787 adult subjects received ziprasidone in all placebo- and active-comparator randomized, controlled studies and had evaluable QTc data. Among these subjects there was not a single instance of QTc ≥ 500 msec or ≥ 480 msec; there were 20 subjects (0.5%) who had a QTc ≥ 450 msec. QTc prolongation ≥ 30 msec was observed in 337 subjects (8.9%); 22 (0.6%) had QTc prolongation ≥ 60 msec; and 5 (0.1%) had QTc prolongation ≥ 75 msec. A plot of peak QTc prolongation as a function of baseline QTc demonstrated that QTc prolongation ≥ 60 msec was not observed in any subject with a baseline QTc ≥ 400 msec. Mean change from baseline to end point in QTc was 3.1 msec (20.31 msec) from a baseline mean of 387.0 msec (21.90 msec). Comparable mean (SD) QTc change values in the pooled placebo ($n = 829$), haloperidol ($n = 865$), olanzapine ($n = 387$), and risperidone ($n = 341$) subjects were -0.6 (20.45), -1.5 (20.77), -1.8 (19.86), and -0.8 (23.09), respectively. Data from pediatric studies; IM studies; and bipolar studies in which ziprasidone was used adjunctively with lithium, valproate, or lamotrigine demonstrate similar QTc changes as those seen in the adult oral studies. From date of first approval through July 31, 2007, 1245 cases of a total of 11,076 reported cases met the MedDRA query criteria for cardiac events. The most commonly reported event was QT prolongation (319 cases) followed by palpitation (146 cases), tachycardia (128 cases), and syncope (127 cases). The ZODIAC trial enrolled 18,154 patients with schizophrenia. The incidence of nonsuicide mortality within 1 year of initiating either ziprasidone ($n = 9077$) or olanzapine ($n = 9077$) pharmacotherapy was 0.91 and 0.90, respectively; the relative risk (95% confidence interval) was 1.02 (0.76-1.39). No cases of torsades de pointes were observed.

Discussion: QTc prolongation has continued to be a major safety concern associated with ziprasidone since before it was launched. This concern notwithstanding, the comprehensive clinical trial data presented here show that the mean increase in QTc caused by ziprasidone is small, that ziprasidone is infrequently ($< 1.0\%$) associated with prolongations of the QTc interval ≥ 60 msec and rarely with a measured QTcF interval ≥ 480 msec. Postmarketing safety reports and the ZODIAC trial provide supportive evidence that ziprasidone is not associated with serious cardiac safety risk in routine clinical practice.

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84. Moderators and Mediators of Cardiometabolic and Treatment Outcomes During the Maintenance Treatment of Bipolar Disorder with Ziprasidone Adjunctive Therapy

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Background: Patients with bipolar I disorder tend to be overweight and at increased risk for metabolic abnormalities. Some studies have

shown obesity and other metabolic illnesses are associated with poor treatment outcomes in such patients. We conducted a post-hoc analysis to test the hypothesis that metabolic syndrome (MetS) and other risk factors are associated with age, gender, race, body mass index (BMI), and symptom severity. We used baseline screening and time course data from a double-blind, placebo-controlled, adjunctive study of ziprasidone plus a mood stabilizer (MS, lithium or valproic acid) in bipolar disorder maintenance treatment. We tested the significance of moderating and mediating effects of MetS status and associated risk factors on symptom reduction as well as the impact of metabolic predictors on relapse rate during the double blind phase.

Methods: The trial comprised 2 phases: a 2.5-4 month, open-label stabilization phase (Phase 1) followed by a 6 month, double-blind maintenance phase (Phase 2). In Phase 1, 584 patients with bipolar I disorder (DSM-IV) received ziprasidone (80-160 mg/d) combined with lithium or valproic acid (ZIP + MS). Patients achieving at least 8 weeks of clinical stability were subsequently randomized and treated with double-blind ziprasidone (up to 6 months) + MS (ZIP + Li, N = 57; ZIP + VAL, N = 70) vs. placebo + MS (PBO + Li, N = 49; PBO + VAL, N = 63). Binary outcomes were investigated using a logistic regression analysis. Relapse rate was analyzed with the Cox regression method and symptom improvement with a multiple regression method. The time courses for weight changes and metabolic parameters over time were analyzed using MMRM method.

Results: Of 1088 baseline screening subjects, overweight/obese (BMI ≥ 25) was found in 65% of patients. Obesity (BMI ≥ 30) was significantly more prevalent in women (42%) than in men (32%) ($p = 0.002$). Among the 584 treated subjects in Phase 1, 482 had fasting laboratory data available for analysis. The majority were female (59%), mean age 38.9 years. Of the 482 subjects, baseline MetS prevalence was found in 111 subjects (23%), 44 in men vs. 67 in women. The prevalence rates were significantly higher in women than men in obesity ($p = 0.002$), abdominal obesity (waist circumference: men > 102 cm, women > 88 cm) ($p < 0.001$), and suboptimal HDL (men < 40 mg/dL, women < 50 mg/dL). High blood pressure was more prevalent in men than women ($p = 0.002$). Subjects with MetS at screening were more likely to be ≥ 40 years old (57% in the MetS vs. 38% in the non-MetS group, $p < 0.05$), had more severe symptoms as assessed by MRS ≥ 18 (79% vs. 69%, $p < 0.05$), had higher abdominal obesity (79% vs. 35%, $p < 0.01$), and higher BMI (95% vs. 55%, $p < 0.01$) (c-statistics = 0.82, $p < 0.001$). During Phase 1 with ZIP + MS treatment, the rate of transition from a normal to a MetS state (incidence rate 9%) is numerically lower than from MetS to normal (13%). We found neutral weight gain and no significant differences in changes in individual metabolic risk factors between the adjunctive (ZIP + Li and ZIP + VAL) groups, with the exception of glucose levels. Fasting glucose was significantly different between the two treatment groups, favoring ZIP + VAL treatment ($p = 0.047$). Increase in abdominal obesity had a negative mediating effect on symptom improvement ($p < 0.05$, as assessed by MRS change score) during Phase 1, while race had a significant moderating effect on symptom improvement (blacks fared worse, and Asians fared better, than whites). In the double-blind Phase 2, the ZIP + MS group had similar weight and metabolic risk profiles compared to the PBO + MS group across all visits, with the exception of greater worsening of HDL at Week 4 in the PBO + MS group. Increase in triglycerides level during Phase 1 was a significant predictor for increased risk of relapse (-2.4, SE 5.7 mg/dL in non-relapse group vs. +25.0, SE 15.7 mg/dL in the relapse group) ($p < 0.05$), defined as intervention for a mood episode. Other MetS associated risk factors were not significant predictors.

Discussion: These results corroborate existing findings on ziprasidone as one of the few antipsychotics exhibiting a neutral weight and metabolic profile in the treatment of psychiatric patients. Our findings suggest that MetS is highly prevalent in bipolar patients and is associated with older age, higher manic symptom severity, and higher BMI. Change in the individual parameters of MetS was also found to mediate treatment outcomes. Given the rising concern of cardiovascular risk, late-onset diabetes and liver diseases associated with MetS,

these results underscore the need for health monitoring and for treatment choices to optimize bipolar treatment outcomes.

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85. Effects of Second Generation Antipsychotics on Peripheral Arterial Compliance

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Background: Treatment with second generation antipsychotics (SGAs) has been associated with adverse metabolic changes such as weight gain, hyperglycemia, and hyperlipidemia. These side effects are also components of the metabolic syndrome, which in turn is associated with increased risk of dire cardiovascular events such as myocardial infarction and stroke. Therefore, there is concern that prolonged metabolic side effects from SGAs could ultimately lead to adverse cardiovascular events. Thus, it is crucial that we increase our understanding of metabolic changes and enhance our prediction of metabolic risk with SGAs in order to prevent such potentially life-threatening events. Arterial compliance (CMP) is the flexibility or the elasticity of arteries. More specifically this can be defined as the change in volume (ΔV) of an artery per unit change in pressure (ΔP): $CMP = \Delta V / \Delta P$. It can be measured in a non-invasive manner using a vasogram, a device that measures volume/pressure relationships to quantify CMP in the large arteries of the leg. The effects of SGAs on CMP are unknown. The objective of this study was to compare CMP in subjects treated with SGAs to healthy controls and to psychiatric controls not on any antipsychotic medication.

Methods: The subject groups consisted of 19 patients treated with risperidone (RISP), 18 on quetiapine (QUET), 27 psychiatric controls who had been off all antipsychotics for at least two months (NOMED), and 111 historical healthy controls (CONT). Subjects were excluded if they were over 70 years old, had diabetes, weight > 300 lbs, or triglycerides > 600 mg/dL. Changes in arterial volume in the thigh and calf across the cardiac cycle were measured with the use of an air plethysmography device called a vasogram. Data on demographic

information, clinical diagnoses, blood pressure, plasma lipid measures, and body mass index (BMI) were also collected.

Results: One-way ANOVAs indicated that the four subject groups differed in BMI and Framingham risk group but not in age or height. A MANCOVA with thigh and calf CMP as dependent variables, subject group as a between-subjects factor, and BMI and Framingham risk group as covariates was used to assess medication effects on CMP. The main effect for subject group was robustly significant for CMP in both thigh ($F(3,169) = 4.61, p = 0.004$) and calf ($F(3,169) = 8.95, p < 0.001$). Follow-up pairwise comparisons for thigh CMP indicated that CONT were higher than RISP ($p = 0.04$) and QUET ($p = 0.001$). For calf, CMP in the CONT exceeded that of RISP ($p = 0.002$), QUET ($p < 0.001$), and NOMED subjects ($p = 0.003$).

Discussion: To our knowledge, this is the first pilot study that has examined CMP in subjects treated with antipsychotics. As hypothesized, subjects treated with RISP or QUET had reduced CMP compared to healthy control subjects (indicating greater cardiovascular risk) independent of the effects of BMI and Framingham risk. The study is limited by the cross-sectional design. Furthermore, there are confounding factors such as dose and duration of prior antipsychotic exposure. The important question of whether our psychiatric subjects had lower CMP independent of drug exposure remains somewhat unclear in this study. The NOMED group had thigh and calf CMP values intermediate between that of CONT and RISP or QUET. These NOMED subjects had been off all antipsychotics for a minimum of two months prior to vasogram testing (16 never treated; mean = 796 ± 1160 days off antipsychotics for the remaining 11 subjects in the NOMED group), but we could not control for periods of medication exposure prior to that time that could have had long-lasting effects on CMP. Reduced CMP has been shown to correlate with coronary and aortic lesions in asymptomatic patients as measured by angiography and MRI. CMP as measured by the vasogram method is reduced significantly in these subjects on RISP or QUET compared to that of healthy CONT. That SGAs may alter CMP has not been reported previously and the mechanism is not understood. CMP measures assessed using the vasogram method may prove useful in assessing the advancement of arteriosclerosis during treatment with SGAs. Larger prospective studies are needed to investigate this potential.

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86. Antipsychotic Medications Regulate Fatty Acid Biosynthesis in Rats: Implications for Hypertriglyceridemia and Metabolic Dysregulation

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Background: Recent *in vitro* studies have found that different antipsychotic medications up-regulate the principal desaturase genes that mediate the biosynthesis of monounsaturated fatty acids (MUFA) and omega-3 (n-3) and n-6 polyunsaturated fatty acids (PUFA). Clinical studies have found that chronic treatment with atypical antipsychotic medications partially normalize erythrocyte docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) deficits in first-episode psychotic patients in association with symptomatic remission. Moreover, chronic exposure to atypical antipsychotics is frequently associated with hypertriglyceridemia and metabolic dysregulation, and stearoyl-CoA desaturase activity, which mediates MUFA biosynthesis, is associated with elevated triglyceride levels and insulin resistance. These and other findings suggest that up-regulation of fatty acid biosynthesis may have implications for the therapeutic as well as adverse effects of antipsychotic medications. To evaluate this phenomenon *in vivo* under controlled dietary conditions, we determined the dose-response effects of five different antipsychotic

medications on PUFA and MUFA biosynthesis in rats, and investigated relationships with non-fasting (postprandial) plasma triglyceride, glucose, and insulin levels.

Methods: Rats received risperidone (RSP)(1.5, 3, 6 mg/kg/d), paliperidone (PAL)(1.5, 3, 6 mg/kg/d), olanzapine (OLZ)(2.5, 5, 10 mg/kg/d), quetiapine (QTP)(5, 10, 20 mg/kg/d), haloperidol (HAL)(1, 3 mg/kg/d) or vehicle for 40 d. Liver Fads1, Fads2, Elovl2, Elovl5, and Scd1 mRNA expression, plasma and peripheral membrane (erythrocyte, heart) fatty acid composition, and non-fasting (postprandial) plasma triglyceride, glucose, and insulin levels were determined.

Results: Chronic treatment with RSP and PAL, but not OLZ, QTP, or HAL, increased liver delta-6 desaturase (Fads2) mRNA expression, and dose-dependently increased both n-3 (plasma 20:5/18:3 ratio) and n-6 (plasma 20:4/18:2 ratio) PUFA biosynthesis. All antipsychotics dose-dependently increased erythrocyte DHA composition, and all except QTP increased erythrocyte AA composition. Only RSP, PAL, and OLZ increased heart DHA and AA composition. RSP, PAL, and QTP dose-dependently increased SCD activity (plasma 18:1/18:0 ratio), but not hepatic Scd1 mRNA expression, and RSP, PAL, OLZ, and QTP treatment dose-dependently increased plasma triglyceride levels. PAL, OLZ, QTP, and HAL dose-dependently increased glucose levels, and no drug significantly altered insulin levels. Among all rats ($n = 122$), SCD activity was positively correlated with plasma triglyceride ($r = +0.8, p < 0.0001$) and glucose ($r = +0.4, p < 0.0001$) levels, and inversely correlated with plasma AA ($r = -0.7, p < 0.0001$) and DHA ($r = -0.4, p < 0.0001$) compositions. Moreover, plasma DHA and AA composition were both inversely correlated with triglyceride and glucose levels.

Discussion: These preclinical data provide *in vivo* evidence that antipsychotic medications modulate fatty acid biosynthesis, and that this mechanism of action is relevant to treatment-emergent hypertriglyceridemia and glucose metabolic dysregulation.

Disclosure: R. McNamara: Part 1; Ortho-McNeil Janssen. Part 4; Ortho-McNeil Janssen. M. DelBello: AstraZeneca, Eli Lilly, Johnson & Johnson, Shire, Ortho-McNeil Janssen, Pfizer, Bristol-Myers Squibb, GlaxoSmithKline. R. Jandacek: None. T. Rider: None. P. Tso: None. A. Strauss: None. J. Lipton: None.

87. Clozapine-induced Locomotor Suppression Is Mediated By The Cortical 5ht2a Receptor In Mice

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Background: Currently available neuroleptic drugs are associated with side effects which range from uncomfortable, to life-threatening. Underlining this inherent problem, findings from the CATIE study (Lieberman, 2005) revealed that up to 74% of patients prescribed atypical antipsychotics (APs) discontinued treatment as a result of either ineffectiveness of the AP or intolerability of the side-effects associated with their treatment. Dissociating the mechanism of therapeutic action of APs from the biological causes of these side-effects is a critical issue facing psychiatric research and is a priority for the development of new targets for the treatment of schizophrenia. First generation APs which predominantly act via blockade of the D2R, are known to be associated with a high risk of extra-pyramidal symptoms (EPS). The subsequent generation of APs, including clozapine, offer a more complex pharmacology which treats the positive symptoms while eliminating the EPS associated with the strong D2R antagonism. The serotonin 2A receptor has been suggested to be critical to the improved efficacy of atypical antipsychotics such as clozapine and this hypothesis is supported by pharmacological studies in animals. However, these drugs come associated with a new cohort of side-effects including weight gain, cardiometabolic risks and sedation. The sedative effects of clozapine have been hypothesized to be a result of its actions at M1, H1 and/or the 5HT2C receptor, but a paucity of evidence to back up these theoretical mechanisms is available. Here, we investigated the role of the serotonin 2A receptor (5ht2AR) in the action of multiple antipsychotic drugs in rodents.

Methods: Wild type and 5ht2AR KO mice were treated with acute haloperidol, clozapine, risperidone and specific 5ht2AR and 2CR antagonists (AC90179 and SB242084) prior to being assessed for behaviors which show some analogy to side effects in humans (locomotor behavior, step down task and righting reflex). Subsequently, selective genetic restoration of the 5ht2AR was used to determine the specific neural population responsible for these behaviors.

Results: Wild-type mice showed a marked reduction in locomotor activity following administration of all 3 antipsychotics (clozapine, risperidone and haloperidol), in addition to severe catalepsy following haloperidol and high dose risperidone, but not clozapine. 5ht2AR KO mice also showed catalepsy following haloperidol and risperidone but did not respond to clozapine's sedative effects in the locomotor arena. AC90179 alone had no effect on locomotor behavior while SB242084 caused locomotor activation. Cortical expression, of the 5ht2AR restored the locomotor suppression caused by clozapine in the open field, but not the righting reflex. Restoration of the expression of the 5ht2AR in dopamine transporter expressing cells had no effect on these behaviors.

Discussion: Catalepsy in rodents is considered to accurately predict EPS in humans. We confirm that, consistent with previously published work, haloperidol and high dose risperidone caused catalepsy in rodents (as measured by the step down task), driven by the strong antagonism of D2 receptors which is present in many first generation neuroleptics. Moreover we rule out a role for the 5ht2AR in catalepsy which has been implied by some reports. We show that mice with no serotonin 2A receptors show no difference relative to WT animals in catalepsy measures following haloperidol and high dose risperidone. Sedation following antipsychotics also has a significant impact on the quality of life of schizophrenia patients. We found that clozapine was responsible for dramatically decreased locomotor activity in wild-type mice in the absence of any notable catalepsy and we hypothesize that this decreased activity may be analogous to the sedative effects of clozapine in human patients. Interestingly, clozapine treated mice had a pronounced loss of muscle tone, just the opposite of the muscle rigidity often associated with D2 blockade induced catalepsy. To our knowledge, serotonin has not previously been considered as a likely target for this locomotor suppression however in the absence of 5-HT_{2A} receptors, mice are not susceptible to this side-effect of clozapine. Moreover, we show that restoring 2A expression in the cortical region restores the ability of clozapine to induce locomotor suppression, suggesting that the cortical serotonin 2a receptors are both necessary and sufficient for locomotor suppressive effects of clozapine.

Disclosure: C. McOmish: None. E. Lira: None. J. Hanks: None. J. Gingrich: None.

88. Maternal/fetal Blood Incompatibility And Structural Brain Anomalies In Schizophrenia

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Background: Prior research has shown that maternal-fetal Rh D and ABO blood incompatibility increase the risk for schizophrenia. In the present study, the relationship between blood incompatibility and volumes of brain structures previously implicated in schizophrenia was assessed in schizophrenia cases and controls from a large birth cohort.

Methods: Cases and controls in the Developmental Insult and Brain Anomaly in Schizophrenia (DIBS) study were drawn from a schizophrenia follow-up investigation of the Child and Health Development Study (CHDS), a large birth cohort. All cases and matched controls were targeted for neuroimaging assessments in adulthood. The DIBS sample consisted of all cases of schizophrenia and other schizophrenia spectrum disorders (SSD) with complete

neuroimaging assessments (N = 26) and 25 matched controls. Cases in the DIBS were similar to subjects in the overall sample with regard to maternal age, race, education and parity. Maternal-fetal incompatibility was assessed by analysis of blood samples in the CHDS birth cohort at the time of the maternal and umbilical blood draws. Exposure to maternal-fetal Rh incompatibility was defined as an Rh-negative (D antigen of Rh) gravida and an Rh-positive fetus. Exposure to maternal-fetal ABO incompatibility was defined as a gravida with blood type O and a fetus with blood type A or B. For the present study, maternal-fetal blood incompatibility was defined as either ABO or Rh incompatibility.

Results: Cases exposed to ABO/Rh incompatibility, compared to unexposed cases, had significantly smaller total cortical gray matter volume ($p = .016$), as well as bilaterally diminished volumes of the dorsolateral prefrontal cortex (DLPFC; right $p = .002$; left $p = .024$) and inferior frontal cortex (right $p = .044$; left $p = .027$). Consistent with these findings, a statistical trend was also observed for increased sulcal CSF volume in exposed cases. In addition, there was a trend for diminished right thalamic volume in exposed cases. In comparison, the ABO/Rh incompatible exposed controls, compared to unexposed controls, did not have smaller total cortical gray matter ($p = .47$) and had reductions in the DLPFC that were not statistically significant; but, similar to cases, had reduced bilateral volume in the inferior frontal cortex (right $p = .014$; left $p = .016$). Exposed controls also had larger hippocampal volume than unexposed controls (total left hippocampus volume $p < .0001$; total right hippocampus volume $p = .01$) and enlarged right putamen volume ($p = .016$).

Discussion: These data suggest that maternal/fetal blood incompatibility may significantly increase the risk for both altered brain morphology and schizophrenia. The findings lend themselves to two main conclusions. First, it is possible that in utero exposure to blood incompatibility heightens the risk for structural brain changes which in turn increase the risk of developing schizophrenia. Second, these data suggest that the exposed controls' enlarged hippocampus may be protective against the development of disease by an adaptive resiliency. Given the modest sample size, further investigation of these results is warranted.

Disclosure: D. Freedman: None. R. Deicken: None. L. Kegeles: None. S. Vinogradov: None. Y. Bao: None. A. Brown: None.

89. Treating Depression After Initial Treatment Failure: A Quasi-Experimental Comparison of Switch and Augmenting Strategies in STAR*D

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Background: Adding or switching to a different antidepressant medication are the most common next-step strategies for depressed patients who fail initial medication treatment, but these approaches have not been directly compared. Our objective was to compare outcomes among patients who received medication augmentation versus medication switching for patients with Major Depressive Disorder (MDD) who did not remit following initial treatment in the Sequenced Treatment Alternatives to Relieve Depression trial (STAR*D).

Methods: We performed a retrospective analysis of STAR*D clinical trial data, which involved both primary care (N = 18) and psychiatric clinics (N = 23) in the U.S. Participants were aged 18-75 with a diagnosis of non-psychotic depression who did not achieve remission with initial citalopram treatment (N = 1,292). Our outcomes of interest were depressive symptom remission, response, and quality of life. Propensity scoring was used to minimize selection bias and allow for augmentation-vs.-switch comparisons.

Results: The propensity score matched augment (n = 269) and switch (n = 269) groups were well balanced on all measured characteristics. Neither the likelihood of remission (Risk Ratio: 1.14, 95% CI 0.82, 1.58)

or response (RR: 1.14, 95%CI 0.82, 1.58), nor the time to remission (Log Rank Test, $p = 0.946$) or response (Log Rank Test, $p = 0.243$) differed by treatment strategy. Similarly, quality of life did not differ. However, post-hoc analyses suggested that augmentation produced better outcomes for patients tolerating 12 or more weeks of initial treatment and those who had partially responded to initial treatment.

Discussion: For patients who received an aggressive initial trial and tolerated it well, there is no clear preference of one strategy over the other. Those who complete an initial treatment of 12 weeks or more and have a partial response with mild depressive severity may benefit more from augmentation relative to switching.

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90. Longitudinal Study of the Diagnosis of Components of the Metabolic Syndrome in Individuals with Binge Eating Disorder

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Background: Binge eating disorder may represent a risk factor for the metabolic syndrome.

Methods: To assess longitudinally the relationship between binge eating disorder and components of the metabolic syndrome, 2.5-year and 5-year follow-up assessments were performed on 134 individuals with binge eating disorder and 134 individuals with no history of eating disorders, frequency-matched for age, sex, and baseline body mass index (BMI). Participants were interviewed regarding new diagnoses of three metabolic syndrome components – hypertension, dyslipidemia, and type 2 diabetes – during the follow-up interval.

Results: In individuals with binge eating disorder vs. comparison individuals, using analyses adjusting for age, sex, baseline BMI, and interval BMI change, the hazard ratios [95% confidence interval] for reporting new diagnoses of metabolic syndrome components were: 2.2 [1.2, 4.2] ($P = 0.023$) for dyslipidemia, 1.5 [0.76, 2.9] ($P = 0.33$) for hypertension, 1.6 [0.77, 3.9] ($P = 0.29$) for type 2 diabetes, 1.7 [1.1, 2.6] ($P = 0.023$) for any component, and 2.4 (1.1, 5.7) ($P = 0.038$) for at least two components.

Discussion: Binge eating disorder may confer risk for components of metabolic syndrome over and above the risk attributable to obesity alone.

Disclosure: J. Hudson: Part 1; Eli Lilly, Pfizer, Otsuka, Alkermes, Ortho-McNeil Janssen Scientific Affairs. Part 4; Eli Lilly, Otsuka, Ortho-McNeil Janssen Scientific Affairs. J. Lalonde: Part 5; Roche. C. Coit: None. M. Tsuang: None. S. McElroy: Part 1; Eli Lilly, Alkermes, Bristol-Myers Squibb, Schering Plough, Sepracor, GlaxoSmithKline, AstraZeneca, Cephalon, Forest, Jazz, Shire, Takeda, Oxigen. Part 4; Alkermes, AstraZeneca, Cephalon, Eli Lilly, Forest, Jazz, Oxigen, Pfizer, Shire, Takeda. S. Crow: Part 1; Eli Lilly, Pfizer, Novartis, Bristol-Myers Squibb, Alkermes. Part 4; Pfizer, Novartis, Bristol-Myers Squibb. C. Bulik: None. M. Hudson: Part 1; Eli Lilly, Pfizer, Alkermes, Ortho-McNeil Janssen Scientific Affairs, Otsuka. J. Yanovski: Obecure Ltd, Roche, Abbott. Part 4; Obecure, Roche, Abbott. N. Rosenthal: Part 5; Ortho-McNeil Pharmaceutical. H. Pope: Part 1; Solvay, Alkermes. Part 4; Solvay.

91. Prenatal Stress And Mood Disorders In The Jerusalem Cohort

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Background: At specific time points in gestation, severe psychological stress from bereavement, war or natural disaster has been linked to increased risk of schizophrenia and autism in the offspring. In a pilot

study of the Jerusalem Cohort we found that the risk for psychiatric disorders other than schizophrenia was increased after in utero exposure to stress in the third month of pregnancy, and that mood disorders made up a substantial portion of these other disorders. In the updated cohort we examined whether severe psychological stress in mothers during the third month of pregnancy significantly increased the risk of mood disorders in offspring.

Methods: Data were analyzed from a cohort of 92,408 offspring born in Jerusalem, followed till ages 33-41 and linked to Israel's national Psychiatric Registry. Data from this cohort were updated and proportional hazards models were used to analyze whether the risk for mood disorders was increased in offspring who were in utero in the third month of gestation during the war of June 1967.

Results: In the updated cohort, after prenatal exposure during the third month of gestation, mood disorders made up 62% of diagnoses other than schizophrenia. The relative risk of hospitalization for mood disorders after intrauterine exposure to war during the third month of pregnancy was 5.54, and the 95% confidence interval was (2.73, 11.24) when compared to risk in all other offspring in the cohort (p value $< .0001$).

Discussion: In this cohort, exposure of pregnant women to an acute, severe psychological stressor in the third month of pregnancy significantly increased the risk of hospitalization for mood disorders in their offspring.

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92. Late-life Depression and Risk of Dementia

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Background: Recent studies have shown that depression is associated with increased risk of cognitive decline, mild cognitive impairment and dementia. Whether depression increases risk of dementing illness or whether late-life depression is a prodrome of dementia remains unclear. To address this question, we examined the associations between dementia and late-life vs early-life depression in participants in the Adult Changes in Thought study, a community-based prospective study of dementia.

Methods: A combined cohort of 3,410 cognitively intact participants aged ≥ 65 years (60% female and mean age-at-entry 74.9 years [65-101]) was followed biennially for incident dementia. Dementia was diagnosed and was classified into Alzheimer's dementia (AD), vascular dementia, mixed, and other type of dementia based on DSM-IV criteria. Depression was assessed at baseline using the 11-item version of the Center for Epidemiologic Studies Depression Scale (CESD-11, total score: 0-33), and defined as CESD-11 score > 10 . Self-reported history and age at onset of depression episodes were collected at the baseline interview. Cox proportional hazards regression with delayed entry and age as the time axis was used to assess the impact of baseline depression (CESD-11 > 10) and depression history on the hazard for dementia.

Results: Over an average of 7.1 (1-15) years follow-up, 658 participants developed dementia, including 386 AD, 89 vascular dementia, 113 mixed dementia, and 70 other type of dementia. Mean age-at-onset of dementia was 83.3 (SD 5.9) years. At baseline, 9% participants had depression with CESD-11 score > 10 and 21% reported a history of depression. Compared with those with no or mild depression (CESD-11 score ≤ 10), the hazard ratio (HR) for all-cause dementia associated with baseline depression was 1.71 (95% confidence interval 1.37, 2.13) after adjusting for age-at-entry (≥ 80 vs. 50) had an increased risk of dementia (adjusted HR = 1.46 [1.16, 1.84]) but those with early-onset depression had no association (adjusted HR = 1.11 [0.83, 1.47]). Compared to participants with neither baseline depression nor early-onset depression history, those with baseline depression without early-life depression are at highest risk for dementia (adjusted HR = 1.77

[1.39, 2.25]). Dementia subtype adjusted HRs associated with baseline depression were: AD, 1.43 [1.05, 1.94]; vascular dementia, 1.78 [0.98, 3.22]; mixed dementia, 2.22 [1.35, 3.65]; and other dementia 2.52, [1.38, 4.64]. Self reported past history of depression at baseline was associated with the other dementia subtype (adjusted HR = 1.80 [1.06, 3.08]) but not with AD, vascular, nor mixed dementia.

Discussion: Association of dementia with late-life depression but not with early-life depression suggests that depression is less likely to be a cause of dementia. Alternatively, late-life depression and dementia may share common brain pathologies, such as cerebrovascular damage, neurodegenerative changes (AD related neuropathological changes and Lewy body pathologies), or late-life depressive symptoms may be a dementia prodrome. The association between depression and dementia does not appear specific to certain clinical subtypes of dementia.

Disclosure: G. Li: None. L. Wang: None. J. Shofer: None. M. Thompson: None. J. Bowen: None. W. McCormick: None. E. Peskind: None. P. Crane: None. E. Larson: None.

93. A Family History Study of Major Depression and Related Disorders in African-Americans Eleanor Murphy*

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Background: Major Depressive Disorder (MDD) is a widespread disorder, currently estimated to be the 4th largest cause of disability and mortality, and which by 2020 is projected to be the 2nd largest cause of disability after Ischemic Heart Disease. Although MDD is shown to be less prevalent among African-Americans compared to whites, Blacks with MDD suffer greater psychological and physical impairment, with longer duration of the disorder (Sommerveld et al., 1989; Kessler et al., 1994; Hassin, et al., 2005; Williams et al., 2007). Furthermore, very little is understood about the patterns of MDD in African-Americans, etiological factors and reasons for the disparity in prevalence rates. Blacks have been underrepresented in family studies that have highlighted very specific patterns of MDD familial aggregation, with early onset and recurrence usually indicative of genetic loadings. Blacks have also been underrepresented in cutting edge genetic linkage and genome-wide association analyses of MDD. In this study, using family history reports, we conduct a preliminary investigation of the familial aggregation of MDD in African-Americans by comparing rates of lifetime MDD in first degree relatives of probands with lifetime MDD among relatives of non-depressed "controls."

Methods: *Participants:* African-American male and female subjects, aged 21 and older residing in the urban New York area, are being recruited to participate in the study. The goal is to obtain data on at least 240 first degree relatives of 20-30 cases with a history of definite MDD, and 20-30 control subjects (matched for age and gender) without a history of MDD or major psychiatric illness. *Measures:* The primary measure used to assess depression in cases is the Diagnostic Interview for Genetic Studies (DIGS) designed to obtain information of lifetime existence of affective, comorbid and exclusionary psychiatric disorders. The Family History Screen (FHS) is designed, through proband reports, to assess the presence of 15 psychiatric disorders among their first-degree relatives. In addition to the clinical and family history interviews, there are two optional self-report measures, a personality inventory and a childhood events questionnaire. *Analyses:* Statistical methods include descriptive analyses and logistic regression models, assessing the rates and relative risk of MDD among relatives of cases compared to that of controls. We will test the hypothesis that relatives of depressed cases are at least twice as likely to suffer from MDD as that of controls.

Results: Preliminary data on 204 first degree relatives to date indicate that for MDD, the rate among relatives of affected probands is 9.5% vs 8.5% for relatives of controls, with a RR of 1.16 (0.37-3.68). For anxiety disorders the rate among affected probands is 17.2% vs. 12.2% for

relatives of controls, with RR of 1.42 (0.55-3.68). For substance dependence, the rate among relatives of affected probands is 21.8% vs. 10.6% among relatives of controls, with a RR of 2.34 (0.86-6.38).

Discussion: The trends from the preliminary findings show elevated rates of substance dependence and anxiety disorders, but not MDD, among relatives of cases compared to relatives of controls. If these trends persist after full data has been analyzed, we may need to consider the possibility that excess of MDD may be masked through substance dependence, which has a high comorbidity with major depression. If the results support the original hypotheses, after controlling for environmental variables, our findings would allow us to generate hypotheses in further family and genetic studies of MDD. Consequently, the findings from this study will be an important step in addressing a critical gap in the current scientific knowledge and understanding of the etiological factors of MDD in African-Americans.

Disclosure: E. Murphy: None.

94. The Neuronal Epigenome of the Prefrontal Cortex : I. Developmental Changes. II. Alterations in Schizophrenia

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Background: There is evidence that epigenetic alterations in prefrontal cortex (PFC) and other brain regions play an important role in the etiology of schizophrenia and other neurodevelopmental disease. Prefrontal neurons permanently exit from the cell cycle during the fetal period, prior to the dramatic changes in functional connectivity, both on a micro- (e.g., synapse) and macro-scale (e.g., network activity, cortical gray matter volumes), that extend into early childhood years and continue throughout adolescence and even beyond. The goal of this study was to obtain a comprehensive and genome-wide map of an epigenetic marking specifically in neuronal chromatin from PFC, including its developmental trajectories and potential alterations in schizophrenia.

Methods: We choose to focus on the trimethylated form of histone H3-lysine 4 (H3K4me3), a type of histone modification linked to the transcriptional initiation complex and activated RNA polymerase II, thereby providing a docking site at the 5' end of genes for chromatin remodeling complexes that either facilitate or repress transcription. Massively parallel (next generation) sequencing from chromatin immunoprecipitates (ChIP-seq) from FACS-sorted NeuN+ nuclei revealed that H3K4me3 is highly enriched within 1-2 KB from annotated transcription start sites (TSS), and is particularly concentrated around TSS associated with CpG islands. Altogether, 32 control subjects across a wide age range (perinatal to old age) and 15 subjects diagnosed with schizophrenia were included in this study.

Results: We present evidence that the neuronal H3K4me3 epigenome of the human PFC is remodeled at many hundreds of loci during the late prenatal period and in the first year of postnatal life, but, unexpectedly, shows comparatively minor changes during subsequent periods of development and aging. From a global, genome-wide perspective, the majority of subjects with schizophrenia displayed an epigenome that was indistinguishable from adult controls, which would suggest that, in these cases, developmentally regulated chromatin remodeling in PFC neurons was not affected by the disease process. Interestingly, preliminary analyses indicates that a subset of schizophrenia subjects (3/15) showed a highly divergent H3K4me3 pattern that differs both from the neuronal epigenomes of the (normal) immature PFC and from those of normal adults.

Discussion: The alterations in the histone methylation landscape in some schizophrenia subjects could reflect either an aberrant trajectory starting with the earliest stages of PFC development, or alternatively, a process unrelated to cortical ontogenesis and maturation altogether.

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95. Gender-specific Association Of A Microcephalin Gene Polymorphism With Brain Volumes In Schizophrenia

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Background: Brain structure and morphology are highly heritable, yet the genetic factors contributing to brain development are mostly unknown. Identification of these factors may lead to identification of the mechanisms underlying abnormal brain development and neuropsychiatric illness. Microcephaly is caused by mutations in a family of genes important in brain development, and evidence is accumulating that common variants may contribute to normal and possibly abnormal brain development. A single nucleotide polymorphism (SNP) upstream of the coding region of the Microcephalin 1 (*MCPH1*) gene (rs11779303) has been previously associated with differences in cranial structural volumes in a sample with and without psychiatric illness (Rimol et al. PNAS, 2010, 107:384-8). These effects were only observed in females. In this work we sought to identify whether any differences in cortical volume were present by rs11779303 genotype in our sample of individuals with schizophrenia.

Methods: A sample of 228 individuals with schizophrenia or schizoaffective disorder and 117 healthy volunteers without psychiatric illness were genotyped at the rs11779303 locus. All individuals have had structural brain MRIs with brain volumes (frontal, temporal, parietal, occipital and total cerebral gray/white matter) measured using automated methods. Analysis of covariance using age, gender, scan protocol, total intracranial volume and diagnostic group as covariates was performed to identify possible associations between brain volumes and genotype. Analyses were also separated by gender, as this difference had been previously reported.

Results: Significant associations by rs11779303 genotype were found with cerebral and parietal gray matter and frontal white matter ($p < 0.05$) in the total sample. When analyzed by gender, these effects were only observed in females. Differences by genotype in cerebral white matter ($p < 0.07$) and frontal gray ($p < 0.08$) approached significance in the total sample. Individuals homozygous for the minor allele (C) had larger gray matter volumes and smaller white matter volumes. The CC homozygotes had smaller total intracranial volumes, but the differences were not statistically significant.

Discussion: Associations of rs11779303 with gray and white matter cerebral volumes were observed in our sample of individuals with schizophrenia and healthy controls, and these effects were specific to females. Replication in additional samples is needed to confirm these preliminary findings. Further investigation is also needed to determine potential effects of this non-coding variant on *MCPH1* gene activity.

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96. Intermediate Neurocognitive and Temperament Phenotypes for Bipolarity in a Genetically Isolated Population

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Background: Investigation of the genetic architecture of intermediate traits associated with bipolar disorder may be a powerful strategy for understanding the genetic basis of this illness. Applying this strategy in rapidly growing genetically isolated populations offers an exceptional opportunity for mapping disease-related loci.

Methods: Here we sought to characterize the familial aggregation and association with disease status of 25 quantitative neurocognitive and

temperament traits selected for their putative association with bipolar disorder (BPD). Study participants included in our analyses to date are 388 members of seven large, multi-generational pedigrees (104 euthymic bipolar I probands and 284 of their non-bipolar relatives; 56% female; age range 18-86 years) ascertained from two closely related genetically isolated populations, the Central Valley of Costa Rica and Antioquia, Colombia. Statistical analyses of familiarity were conducted using the FCOR procedure in SAGE (Statistical Analysis for Genetic Epidemiology), after adjusting for significant covariates. FCOR assesses the correlations between parents and offspring (P-O) and among sibling pairs. SPSS software was used for tests for association with BPD. Correcting for multiple comparisons, we set the threshold for statistical significance at $p = .002$.

Results: Of the traits examined, verbal cognitive abilities (verbal IQ, verbal working memory and verbal fluency) showed the highest familial aggregation, with sibling correlations of .37, .35 and .25, equivalent to heritability estimates of 74%, 70%, and 50%, respectively (all $p < .001$). P-O correlations were also significant for these traits ($p < .002$). In the temperament domain the highest familial aggregation was seen for delusion proneness, as assessed by the Peters Delusion Inventory (PDI; sibling and P-O correlation = .27, $h^2 = 54\%$; $p = .001$), and attentional impulsivity, as measured by the Barratt Impulsivity Scale (BIS; sibling and P-O correlation = .28 and .29, respectively; $p = .001$). Perceptual creativity, assessed by the Barron-Welsh Art Scale, also showed near-significant sibling correlations ($r = .24$; $p = .003$) in the pedigrees, but P-O correlations did not reach significance ($r = .12$; $p = .20$). Bipolar I probands performed similarly to their non-bipolar relatives on Verbal IQ, abstraction, and verbal working memory measures, but had poorer performance on measures of verbal fluency (Animal Naming: 2.77 fewer items, on average), sustained attention (Continuous Performance Test: 8.01 fewer hits, on average), and slower processing speed (Trails A: 29.74 seconds slower, on average; Trails B: 49.77 seconds slower, on average). Bipolar probands also had more extreme scores than their non-ill relatives on measures of impulsivity, cyclothymic temperament, and delusion-proneness, although relatives of bipolar probands also showed elevated scores relative to normative values on these measures.

Discussion: To our knowledge this is the first family-based study of familial aggregation of endophenotypes for bipolar disorder in a genetically isolated population. Findings suggest that these cognitive and temperament traits offer promise for further study as quantitative traits associated with the genetic basis of bipolar disorder. In subsequent analyses we will apply the traits demonstrating the highest familial aggregation to combined genome-wide linkage and association analysis.

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97. Rare ANK3 Gene Sequence Variants in Bipolar Disorders

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Background: Bipolar disorder (BPD) is a common chronic, often disabling group of illnesses affecting ~2% of US adults. Twin studies indicate that the majority of risk for BPD is inherited. In the past two years, multiple genome-wide association studies (GWAS) have detected risk haplotypes (odds ratio ~1.3) in the ankyrin 3 gene (ANK3) on chromosome 10 at ~61,500,000 bp (Ferreira et al, Nat Genetics, 2008; Scott et al, PNAS, 2009; Schulze et al, Mol Psychiatry, 2008; Smith et al, Mol Psychiatry, 2009). ANK3 protein (Kordeli et al, JBC, 1995) chaperones ion channel subunits, so that they are properly oriented into the neuronal cell membrane. The strongest association

signals vary widely across the ~700 kb DNA fragment comprising the ANK3 gene for these studies, an observation consistent with substantial allelic heterogeneity. Given the brain-specific nature of BPD signs and symptoms, a single 8 kb serine-rich ANK3 exon (the expression of which is limited to the CNS), was selected for sequencing, using ~400 DNA samples from BPD persons participating in NIMH-sponsored genetic studies (Smith et al, Mol Psychiatry, 2009). This experiment was designed as a search for rare variants in ANK3 which might increase risk for BPD.

Methods: From ~10 ng genomic DNA of ~400 BPI individuals, the 8 kb exon was PCR-amplified (using a SequelPrep kit) and either treated with ExoSAPit® or gel purified. 16 pools of 24 amplicons each were created. These pools were dried down, resuspended in 100 µl nuclease-free water, sonicated in a Covaris single cell bath sonicator for 6 minutes between 4-7 °C, followed by end-repair, ligation to bar-coded P2 (to permit pool identification) and P1 sequencing primers; library amplification, size selection and gel purification were done according to the manufacturer's recommendations. Purified, barcoded libraries were quantified by qPCR and then clonally-amplified by emulsion PCR. Sequencing was done in an ABI SOLiD machine, which uses a sequencing by ligation technology. ~6.4 million ~50 bp 'mappable' reads were generated from each pool, totaling ~5000 Mb of sequence, of which ~95% mapped to the target ANK3 exon. Burrows-Wheeler alignment tool (Li and Durbin, 2009) was used to align sequences to the genome. Modified software of Holt et al (2010) and custom programs were used for variant detection. Variants which changed the amino acid sequence at an evolutionarily conserved residue were selected for confirmation by RFLP or Sanger sequencing. **Results:** Rare SNPs listed in the databases (dbSNP, SeattleSNPs) were detected and validated (rs3134609, rs3802696, rs41274674, rs10740006, rs41274672, rs28932171, rs10821668, rs7923682), lending confidence to the SNP-calling methods. At least 10 mis-sense variants (previously unreported) at conserved amino acid residues were detected and confirmed in this ongoing analysis, including D2319N, F2375V, E3563G and ΔE1926.

Discussion: These mis-sense variants may contribute to BPD susceptibility and are currently being genotyped in ~4000 BPI DNAs from the NIMH Genetics Initiative and ~4000 NIMH screened controls, to estimate allele frequencies in these populations. Targeted sequencing of genes in which common alleles are implicated in BPD may reveal uncommon variants which increase risk.

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98. An HTR2B Low-Expression Haplotype Predicts Risk of Cocaine Dependence and Aggression

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Background: Recently we identified a stop codon in the HTR2B gene that is restricted to Finnish Caucasians. It was associated with impulsive aggression in a case-control and family-based segregation analysis. Impulsivity and aggression are important features of several psychiatric diseases, including borderline personality disorder (BPD) and addictions. Impulsivity is a predictor of cocaine use in rats and relapse in humans. To investigate the role of HTR2B in other populations we analyzed an African-American population characterized by cocaine dependence and a sample of BPD cases and controls with a behavioral measure of aggression and brain function using PET imaging. We linked our findings in these two samples with haplotype-dependent protein expression in lymphoblasts. We had previously demonstrated by qPCR that HTR2B is widely expressed in human brain.

Methods: A sample of 382 African-American men was recruited from the Substance Abuse Treatment Program at the Department of Veteran

Affairs New Jersey Health Care System: 208 men with lifetime DSM-IV diagnoses of cocaine dependence and 174 controls. Four haplotype-tagging HTR2B SNPs (rs6736017, rs6437000, rs1549339, rs17586428) were genotyped together with 186 ancestry informative markers. Single SNP and haplotype analyses were performed. All statistical results were Bonferroni-corrected. The HTR2B rs6437000 (T/G) SNP was analyzed in a sample of 32 participants with DSM-IV BPD and 39 matched controls. Aggressive behavior was assessed using the Point Subtraction Aggression Program (PSAP). A subsample of 17 cases and 25 controls underwent 18FDG PET imaging while performing PSAP. Relative glucose metabolic rates were assessed in 39 Brodmann areas (BAs) and the amygdala in each hemisphere by coronal slice tracing. Results were controlled for gender, age and self-reported ethnicity. Findings in these two samples were compared with haplotype-dependent protein expression data generated by Western blot in 12 lymphoblasts from our earlier study in Finnish Caucasians.

Results: In the African American sample, there were 4 common haplotypes. Two haplotypes conferred increased risk (O.R. = 1.5) for cocaine dependence; the other two were protective (O.R. = 0.6) (global $p = 0.005$). SNP rs1549339 ($p = 0.008$, 2df) and rs17586428 ($p = 0.02$, 2df) were both associated with cocaine dependence. In the BPD sample, the rs6437000 T allele carriers performed more aggressively than GG homozygotes in both patient and control groups ($F(1,71) = 6.09$, $p = 0.016$). In the PET imaging subsample, T allele carriers showed higher activation with aggression provocation compared to GG homozygotes in BAs 44 and 20 (left hemisphere) ($p < 0.05$). Additionally, BPD patients carrying the T allele showed a higher activation in the left amygdala compared to GG homozygotes ($p < 0.01$). We had previously measured protein levels in 12 lymphoblastoid cell lines from Finnish Caucasians heterozygous for the HTR2B stop codon and 12 homozygous non-carriers. Predictably, protein expression was halved in the cell lines heterozygous for the stop codon ($p = 0.003$). However, of these 12 heterozygous cell lines, five carried a haplotype that in the African Americans was associated with cocaine dependence and five carried a haplotype that was protective. These risk and protective haplotypes were correlated, respectively, with significantly lower and higher 5-HT_{2B} receptor levels ($p = 0.008$). Moreover, the rs6437000 T allele that in the BPD sample was associated with greater aggression, was included in the low activity haplotype.

Discussion: This study implicates HTR2B in aggressive behavior and cocaine dependence in two independent samples. Moreover, the haplotypes associated with behavior appear to predict 5-HT_{2B} protein levels in lymphoblasts. Thus there may be both rare (a recently described stop codon) and common (a locus influencing expression) functional variation at HTR2B.

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99. Genetics of GRM7 in Schizophrenia and Bipolar Disorder

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Background: GRM7, the gene that codes for the metabotropic glutamate receptor mGlu₇, has been previously identified as a possible risk gene for schizophrenia and bipolar disorder. We identified a series of SNPs in GRM7 that are associated with schizophrenia in the Clinical Brain Disorders Branch sample, and/or bipolar disorder in the Mood and Anxiety Disorders Program sample, respectively.

Methods: To determine if these SNPs have functional implications, we tested their effects on neural circuitries related to mental illness as measured by functional MRI in healthy adults. The first fMRI task was

a working memory task, the N-back, which has been shown to detect cortical inefficiency in patients with schizophrenia. The second task was an emotional memory task, which activates the hippocampus and amygdala, which have been shown to be altered in bipolar disorder. In order to understand the biology underlying the functional differences, we then measured GRM7 mRNA expression in a post-mortem brain sample. Gene expression was measured in the dorsolateral prefrontal cortex of 283 brains from normal controls using 4 different probes in GRM7, each marking a unique mRNA transcript. The samples were collected from individuals ranging in age from second trimester fetus to 83 years (average RNA integrity number = 8.34, post-mortem interval = 26 h, pH = 6.52).

Results: We found that carriers of a schizophrenia risk allele (rs1810320) had greater activity in the dorsolateral prefrontal cortex (less cortical efficiency) during the 2-back compared to the 0-back than the subjects that were homozygous for the common allele ($p < 0.05$, FDR corrected for whole brain). Carriers of the risk allele for bipolar disorder (rs13071462) had increased amygdala and hippocampal activation during the emotional faces task ($p < 0.05$, FDR corrected for ROI), and also had increased activation in the dorsolateral prefrontal cortex during the N-back (p -uncorrected < 0.001 , whole brain). The schizophrenia risk SNP (rs1810320) predicted age-adjusted expression of a novel GRM7 transcript measured by Illumina oligonucleotide probe 34943 (ANCOVA $p = 8.15 \times 10^{-5}$). The bipolar risk SNP did not predict expression of any of the measured GRM7 transcripts.

Discussion: In summary, we identified SNPs in GRM7 that are associated with schizophrenia or bipolar disorder. The schizophrenia risk SNP predicts a particular GRM7 transcript that increases substantially during fetal development, peaks at birth, declines throughout childhood, and remains very low in adulthood, suggesting that it plays a role in brain development. Therefore GRM7 may modulate risk for schizophrenia by altering gene expression during fetal development, which results in altered brain function as measured by fMRI. The bipolar risk SNP was not associated with any of the measured GRM7 SNPs, however there are likely many more novel transcripts that have yet to be described. It may be that the bipolar risk SNP alters a different transcript that has a different expression profile across the lifespan. Further research into the mechanism of risk is necessary for both schizophrenia and bipolar disorder, but these data suggest that mGluR7 may be a promising target for therapeutics in these illnesses.

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100. Long Term Effects of Early Trauma on FKBP5 Gene Expression and Glucocorticoid Receptor Sensitivity are Genotype-Specific

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Background: FKBP5, a co-chaperone of hsp90, regulates GR sensitivity by an ultra-short feedback loop and this regulation is dependent on specific functional polymorphisms. A number of studies have now reported that these functional polymorphisms moderate the impact of early trauma on the development of psychiatric disorders, including post traumatic stress disorder (PTSD), suicide attempts and depression. The molecular mechanisms by which this might be mediated have, however, not been elucidated yet.

Methods: In a cohort of 277 individuals with high levels of trauma, we investigated baseline cortisol levels as well as the response to a low-dose dexamethasone (0.5 mg) suppression test. In 235 individuals FKBP5 mRNA expression was analyzed from whole blood. Child abuse was assessed using the trauma experience inventory (TEI) and 35.7% reported at least one type of child abuse. Current depression was

assessed using the Beck depression inventory (BDI) and PTSD symptoms using the PTSD symptom scale (PSS). Analyses included child abuse severity and FKBP5 rs9296158 genotypes as predictors.

Results: We observed the previously reported positive correlation of FKBP5 mRNA expression and serum cortisol levels (Pearson $R = 0.45$, $p < 0.001$) which reflects the induction of FKBP5 transcription by GR activation within the feedback loop. This correlation was significantly attenuated in individuals exposed to early trauma. While the correlation was $R = 0.50$, $p < 0.001$ in individuals without early trauma, it was only $R = 0.149$, $p > 0.05$, in individuals exposed to 2 types of child abuse. This same analysis was then repeated after stratification by the rs9296158 genotype. The child abuse-dependent disruption was only observed in carriers of the rs9296158 risk allele but not carriers of the protective GG-genotype, where the correlation coefficients were identical in the severely abused vs. non-abused group. This disruption of the FKBP5-dependent regulation of GR function was also reflected in differences in GR sensitivity as measured by the low-dose dexamethasone suppression test. We observed a significant triple interaction of time, level of child abuse and rs9296158 genotypes ($p = 0.036$) and interaction between child abuse and rs9296158 ($p = 0.005$), controlling for age, gender and current depression and PTSD symptom severity on serum cortisol levels in this test. Exposure to child abuse led to an enhanced dexamethasone suppression and thus increased GR-sensitivity only in carriers of the A-risk allele, paralleling the allele-specific disruption of GR-induced FKBP5 regulation.

Discussion: The FKBP5 genotype-dependent moderation of the impact of child-abuse on psychiatric symptoms might be mediated by genotype-specific effects of child abuse on GR-sensitivity regulation by FKBP5. The ultra-short feedback loop between FKBP5 and GR appears to be disrupted only in severely abused individuals with the FKBP5 risk genotype. This could involve allele-specific epigenetic changes and this hypothesis is currently being investigated.

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101. Identification of Children and Adolescents At-Risk For Psychosis: Results of a Screen for Sub-Psychotic Symptoms in a Community Sample

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Background: Accurate identification of youth at-risk for psychosis will require a combination of clinical, neurobehavioral, and genetic risk indicators. While many investigations aim to identify at-risk youths among the clinically help-seeking, a complementary approach entails community-based screening for more comprehensive identification. Screening in community samples requires brief and efficient tools that are sensitive, specific and reliable in children and adolescents. The PRIME Screen is a 12-item screening questionnaire developed to assess sub-psychotic symptoms (Miller et al. 2004). Prior work has shown high sensitivity and specificity in young adult clinical (Miller et al. 2004) and non-clinical (college student) samples (Kobayashi et al. 2008). For reasons both developmental and methodological, we cannot assume that the successful application of a screening tool in young adults will generalize to children and adolescents. One study in adolescent students ($n = 532$) reported that 18.4% endorsed one or more sub-psychotic symptoms (Fresan, 2007). To our knowledge, there have been no investigations of the properties of the PRIME Screen in a community sample of children and adolescents. The current investigation evaluated the generalizability of the PRIME Screen to a large sample of children and adolescents and its suitability for studies examining relationships among clinical, neurobehavioral and genetic risk indicators of psychosis.

Methods: Children (age 11-21; $n=743$; M:F=322:421) participated in the Neurodevelopmental Genomics and Trajectories of Complex Phenotypes (NGTCP) project, a study aiming to characterize clinical and neurobehavioral phenotypes in a prospectively accrued community cohort of 10,000 children who have been genotyped using high-density SNP arrays. In the context of a comprehensive psychopathology screener, participants completed an assessor administered PS-R (PRIME Screen-Revised, Kobayashi et al., 2008). Items are self-rated on a 7-point scale ranging from 0 (Definitely Disagree) to 6 (Definitely Agree); a second rating scale requires a rank of first onset of each endorsed symptom. Using the original PRIME Screen, a positive screen is >1 item rated 6. Kobayashi et al. incorporated duration criteria (>1 year) to reduce false-positives; however, current at-risk approaches tend to place high significance on recent symptom onset. Consequently, participants in the current investigation were divided into 3 groups: Negative=No items rated 6; Positive-Acute (≥ 1 item rated 6 with onset in past year); Positive-Chronic (≥ 1 item rated 6 but none with onset in past year). PS-R Total score was also calculated to provide a quantitative risk indicator.

Results: Internal consistency of the PS-R was high (Cronbach's $\alpha=.85$), and similar to prior reports. Without regard to symptom duration, 14.6% ($n=111$) of participants rated 6 for ≥ 1 item. Among the Positive groups, a greater number were Chronic (8.4%; $n=63$; mean age=14.8, PS-R Total mean=25.5) than Acute (4.9%; $n=37$; age=15.2; PS-R Total=22.1). The Negative group (86.7%; $n=650$; mean age=16.3; PS-R Total=5.6) had a lower PS-R Total (ANOVA, $p<.001$), and was significantly older than both at-risk groups (ANOVA, $p<.001$). The PS-R total had a small but significant correlation with age (Pearson $r=-.20$, $p<0.001$), indicating that older participants tended to endorse fewer or less severe items than younger participants. Stratification at age 15 and above ($n=412$) and below age 15 ($n=164$) revealed that there was a disproportionately higher number of Negatives among the older (88.3%) than the younger group (78%; Chi-square, $p<.0001$).

Discussion: This preliminary study supports further investigation of the PS-R as a screening tool for sub-psychotic symptoms in a community sample of children and adolescents. Differences across ages in endorsed symptoms may reflect true differences in symptom prevalence or instead a methodological artifact such as developmental differences in comprehension of PS-R screen items. Similarly, estimates of symptom duration may be influenced by cognitive development. Further work will entail investigating the concordant validity of the PS-R as a tool for early identification of at-risk youths through follow-up of PS-R Negative and Positive individuals, and longitudinal examination of reported symptoms in relation to conversion to psychosis. Our DNA repository, neuro-cognitive and imaging data will enable us to evaluate these characteristics as predictors of risk status, and perhaps ultimately improve early identification of those at highest risk of developing psychosis.

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102. Genome-Wide Expression and SNP Microarray Data Implicate PCLO Gene in Bipolar Disorder

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Background: Genetic variation may contribute to differential gene expression in individuals with psychiatric disorders. We explored potential association between genes differentially expressed in the prefrontal cortex (PFC) of individuals with bipolar disorder and single nucleotide polymorphisms (SNPs) adjacent to those genes.

Methods: We used genome-wide expression and SNP microarray data of bipolar disorder ($N=40$) and unaffected controls ($N=43$) from the Stanley Genomics Database. We identified 367 genes as being differentially expressed (fold change >1.3 and FDR-adjusted q -value <0.05) in the PFC of bipolar disorder. We then identified local SNPs (100 kb up- and down-stream of each gene) that are associated with expression levels of those genes (FDR q -value <0.05). Using those local SNPs, we tested disease association with the results derived from a meta-analysis of genome-wide association studies (GWAS) including 4,936 bipolar disorder subjects and 6,654 healthy controls.

Results: We identified 45 gene and SNP associations in the PFC of bipolar disorder (FDR q -value <0.05). Several genes such as HBS1L (15 SNPs), HLA-DPB1 (15 SNPs), AMFR (8 SNPs), PCLO (2 SNPs) and WDR41 (2 SNPs) showed association with multiple SNPs. A SNP (rs13438494) in an intron of the piccolo (PCLO) gene was significantly associated with bipolar disorder (FDR-adjusted $p<0.05$).

Discussion: Current results support the previous findings implicating PCLO in mood disorders and demonstrate the utility of combining genomic and transcriptomic data to enhance our understanding of the genetic contribution to bipolar disorder.

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103. Do Interactions Between Family Stress and the 5HTTLPR Polymorphism Predict General Psychopathology in Youth at Risk for Pediatric Bipolar Disorder?

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Background: Offspring of parents diagnosed with bipolar disorder are at increased genetic risk for developing bipolar disorder and other types of psychopathology. Lower levels of family cohesion and/or organization as well as increased levels of family conflict have been associated with family environments of bipolar offspring. For offspring of bipolar parents, life stressors such as poor family functioning may both hasten the onset and increase the incidence of psychopathology. Reports have demonstrated that the influence of life stressors, such as poor family functioning, on psychopathology may be moderated in part by genetic vulnerability. Specifically, presence of multiple short (S) alleles of the 5HTT-linked polymorphic region of the serotonin transporter (5HTTLPR), a relevant mediator of serotonin uptake into presynaptic neurons, may increase risk of mood episodes in response to life stressors. The objective of this study was to determine whether 5HTTLPR genotype moderated the influence of poor family functioning on levels of general psychopathology in bipolar prodromes and/or healthy controls.

Methods: Bipolar prodromes (PD; $n=24$) and healthy controls (HC; $n=21$) were genotyped for the 5HTTLPR polymorphism and assessed with the self-report Family Adaptability and Environment Cohesion Scale (FACES). Parents of participating youth completed the Child Behavior Checklist (CBCL). The four "unbalanced" FACES scales (disengaged, enmeshed, rigid, chaotic) were summed to form one "unbalanced" variable for analyses. Backward stepwise regression for 5HTTLPR was conducted to determine the best predictive model where 5HTTLPR genotype, "unbalanced" FACES score, group (PD or HC), and their interactions served as independent variables and CBCL total raw score as the dependent variable.

Results: PD and HC groups did not differ significantly on 5HTTLPR genotype frequencies (Chi-square = 3.67, $df=2$, $p=.16$). PD youth had significantly higher CBCL total raw scores ($t=8.49$, $df=43$, $p<.01$) than HC youth. There was a modest trend where PD youth reported slightly higher levels of "unbalanced" family functioning ($t=1.99$, $df=43$, $p=.05$). For 5HTTLPR, a group \times genotype \times "unbalanced" family functioning interaction ($F=29.79$, $df=1$, $p<.001$) was a significant predictor of CBCL Total score. PD youth with an L/L genotype and poor family functioning were more likely than PD youth

with S,L and S,S genotypes and poor family functioning to have high CBCL Total scores. Genotype did not significantly moderate the relationship between poor family functioning and general psychopathology in healthy controls.

Discussion: Youth at high risk for development of bipolar disorder, with L,L 5HTTLPR genotypes and living in poorly functioning family environments, showed higher levels of general psychopathology than PD youth with S,L or L,L genotypes. This finding contradicts previous findings implicating the S allele as a relevant moderator of the impact of life stress on psychopathology. L,L 5HTTLPR genotype, coupled with poor family functioning, may place offspring of bipolar youth at risk for development of psychopathology. A number of studies have focused on the influence of an interaction between the S allele and early childhood adversity on individuals with depression. The L allele may interact with different life stressors such as poor family functioning to confer greater risk to the development of other psychopathologies such as bipolar disorder. Future gene x environment investigations should focus on interactions between additional life stressors and 5HTTLPR genotype to determine their effect on the development of psychopathology. Results from this pilot are limited by sample size, and replication in a larger sample is warranted.

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104. Genetic Studies of the Acute Response to Amphetamine: An Intermediate Phenotype Approach

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Background: Both the subjective effects of drugs and the risk for drug abuse are heritable traits. Sensitivity to the subjective effects of drugs is a risk factor for drug abuse. Based on this observation, we and others have hypothesized that the genetic polymorphisms that modulate the subjective effects of drugs may also influence the risk for drug abuse. For these reasons we consider sensitivity to the subjective effects of drugs an intermediate phenotype for drug abuse.

Methods: We have measured the subjective, behavioral, and physiological response to acute doses of amphetamine (0, 10 and 20 mg) in almost 400 healthy young adults. We used sparse factor analysis to extract a small number of factors that capture the bulk of the observed phenotypic variability in these data. We have performed both candidate gene and genome-wide association analyses on both traits and factors, employing a Bayesian approach to the latter.

Results: We have identified multiple associations using both the candidate gene and genome-wide association approaches ($\log(\text{Bayes Factor Scores}) > 4$ is considered suggestive for the genome-wide analysis).

Discussion: The results allow us to identify alleles that influence different components of the acute response to amphetamine and may complement genome-wide association studies of risk for stimulant abuse or other clinical phenotypes. This research was supported by R01DA021336, R01DA02812, R03DA027545 and T32DA007255.

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105. Traversing the Boundaries of the DSM-IV: The Genetics of Symptom-Based Phenotypes

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Background: Molecular genetic studies increasingly suggest that genes do not respect the boundaries of the current diagnostic system.

For example, several well known candidate genes have been linked to multiple diagnostic groups including schizophrenia, schizoaffective disorder, bipolar disorder and major depressive disorder. Although efforts to identify quantitative trait loci (QTLs) associated with the symptom dimensions of major psychiatric disorders have met with early success this analytic approach has been limited to specific diagnostic groups. Thus, data seeking to elucidate the basis of common genetic risk across diagnostic groups are extremely limited.

Method: We genotyped a multi-diagnostic group of 764 patients with psychosis and 193 healthy individuals using a custom Golden Gate Illumina 1536 SNP chip. The chip was designed to comprehensively assess several genes, including *NRG1*, *ERBB3*, *CACNA1C*, *ZNF804A* and *ANK3*, which showed prior association to multiple diagnostic groups. Using lifetime ratings of psychosis we then assessed the relationship between genetic variation at these loci and phenotypic variation without regard to diagnostic group membership.

Results: We identified several associations to symptom domains in each of these genes. After correction for multiple testing, however, only SNPs in *ANK3*, *ZNF804A* and *NRG1* remained significant. In each case, the significant association was to a lifetime history of hallucinations. Follow up analyses indicated that none of these SNPs were significantly associated with case-control status in either the full group or in any individual diagnostic group.

Discussion: The present results suggest that delineation of the role of specific genes on symptom dimensions, rather than diagnostic entities is possible. Moreover, such methods may lead to more refined approaches to understanding the etiology of psychiatric disorders characterized by psychosis and may suggest novel treatment targets for specific domains of illness.

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106. Chronic Alcohol and Cocaine Exposure in the Human Hippocampus: Global Effects on Gene Expression and Specific Effects on GABAergic Genes

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Background: Neuronal adaptation underlies addiction to alcohol and cocaine and results from widespread cellular and molecular changes in the brain. The hippocampus is a key brain region involved in both short-term and long-term learning and memory processes and may play a critical role in drug-associated behaviors including craving and relapse. In this study we undertook whole genome sequencing of mRNA transcripts in the body of the hippocampus (dentate gyrus and Ammon's horn) using RNA-Seq, a methodology that provides precise, accurate measurements of the level of transcripts and their isoforms. We first analyzed up- and down-regulation of expression across all 20,000 genes that had identifiable transcripts and then focused on genes within the GABAergic system that is implicated in addiction and long-term potentiation (LTP) within the hippocampus. We sought to identify gene expression changes in the hippocampus that may underlie all addictions as well as changes specific to alcohol and cocaine dependence.

Methods: Samples of postmortem hippocampus were obtained from the University of Miami Brain Endowment Bank from 8 alcoholics, 8 cocaine addicts and 8 controls. The total sample consisted of men who had died suddenly at a mean (SD) age of 38.1 (6.9) yrs. There was no difference in mean age, ethnicity or postmortem interval between the three groups. Total RNA was extracted, mRNA was isolated and high-throughput parallel sequencing was performed across the whole genome using the Illumina Genome Analyzer. Approximately 346 million bases (9.6 million sequence reads, length 36 bp) were mapped per sample. The read counts were then log₂ transformed and normalized using quantile normalization before group comparisons were performed.

Results: Genome-wide, RNA-Seq changes associated with alcohol and cocaine addiction were extensive, and included both up- and down-regulation. Changes specific to chronic cocaine use were found in genes responsible for mitochondrial oxidative phosphorylation, energy metabolism and LTP. The effect of chronic alcohol and cocaine exposure was predominantly to significantly decrease gene expression across the presynaptic, synaptic and postsynaptic GABAergic pathway. Cocaine addicts had reduced expression of genes GAD1, GAD2 and ABAT that respectively encode for presynaptic GABA synthesis and metabolism. In both cocaine addicts and alcoholics, GAT1 (GABA transporter) and GABBR1 (presynaptic autoreceptor) were down-regulated, possibly resulting in increased GABA in the synaptic cleft. Chronic cocaine and alcohol exposure resulted in downregulation of GABRG2 that encodes the gamma2 subunit that is included in nearly all GABAA postsynaptic receptors. Chronic cocaine and alcohol exposure also had effects on genes encoding GABAA receptor-associated trafficking proteins, principally GABARAP, NSF and gephyrin. Finally, chronic alcohol exposure was associated with downregulation of GABRA2 and the closely adjacent GABRG1. Variation in both of these genes has been robustly associated with alcoholism in case-control studies.

Discussion: This study illustrates a method for identifying neuroadaptations in response to chronic alcohol and cocaine exposure both globally, across 20,000 genes, and within specific candidate pathways. Our results demonstrate that chronic, heavy alcohol consumption and cocaine abuse is likely to result in changes that are both specific to alcohol or cocaine addiction and common to both addictions, at least within the hippocampus.

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107. Association of SNPs in Genes Related to the Neurosteroids Pathway with Anxiety, Panic Disorder and Depression

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Background: Neuroactive steroids are synthesized in the brain tissue (neurosteroids) from cholesterol or steroidal precursors. Neurosteroids have been shown to be implicated in the neural proliferation, differentiation and activity. Recent preclinical and clinical studies also suggest a modulatory role of neurosteroids in anxiety and depression. Rupprecht et al. (2009) described an anxiolytic effect of the substance XBD173, which binds to the mitochondrial benzodiazepine receptor (TSPO) thereby promoting neurosteroid synthesis.

Methods: In order to investigate the genetic role of the genes from neurosteroid pathway, we performed an association study in patients with anxiety disorders and depression. We selected 5 genes mainly from the biosynthesis pathway, which were steroid-5-alpha-reductase 1A (SRD5A1), aldo-keto reductase family 1 C1-C3 (AKR1C3-AKR1C3), and the TSPO gene. A total number of 49 SNPs was genotyped in 280 patients with anxiety and panic disorders, 350 depressed patients and 500 healthy controls.

Results: First case-control results suggest associations of rs39848/rs4702371 (promoter region of SRD5A1), rs11744535 (downstream SRD5A1) and rs11592008 (intronic, AKR1C3) with both anxiety disorders and PD.

Discussion: This is the first preliminary evidence for the implication of genes from neurosteroid synthesis pathway with the anxiety disease-status phenotype. Further statistical test and analysis of dimensional anxiety phenotypes are ongoing to validate these first associations.

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108. Serotonin Transporter Gene Functional Polymorphisms and Susceptibility to Bipolar Disorder: An Updated Meta-Analysis of Association Studies

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Background: The serotonin transporter gene (SLC6A4) has long been considered a promising candidate gene for neuropsychiatric disorders, especially mood disorder, based on serotonin transporter's crucial role in serotonergic neurotransmission. However, association studies have produced conflicting results regarding the association between two common functional SLC6A4 gene polymorphisms, the promoter insertion/deletion (5-HTTLPR) and the intron 2 Variable Number Tandem Repeat (STin2 VNTR) polymorphisms, and bipolar disorder susceptibility.

Methods: To further elucidate the putative association between the two SLC6A4 gene functional polymorphisms and bipolar disorder susceptibility more comprehensively, we performed an updated meta-analysis based on all original published association studies between the 5-HTTLPR, STin2 VNTR polymorphisms and bipolar disorder. MEDLINE (using PubMed), Web of Science and reference lists were systematically searched through August 2010. Three primary criteria were used to judge whether a study could be included in the meta-analysis. Overall, 50 and 26 association studies met criteria for inclusion were identified for the 5-HTTLPR and STin2 VNTR polymorphisms respectively. Meta-analysis was conducted using the DerSimonian-Laird random-effects model. The method described previously by Lohmueller et al (2003) was utilized to calculate the combined effect size from both case-control and family-based association studies.

Results: For the 5-HTTLPR polymorphism: meta-analysis showed no statistically significant evidence for association between the Short allele and bipolar disorder (pooled odds ratio (OR)=1.05, 95% Confidence Interval (CI)=0.99-1.11, Z=-1.8, P=0.075), and homogeneity analysis found no statistically significant evidence for heterogeneity of the ORs among the group of studies. For the STin2 VNTR polymorphism: meta-analysis showed no statistically significant evidence for association between the STin2.12 allele and bipolar disorder (pooled OR=1.07, 95% CI=0.97-1.17, Z=1.4, P=0.17). However, homogeneity analysis found statistically significant evidence for heterogeneity of the ORs among the group of studies, and meta-analysis of STin2 VNTR polymorphism was performed after exclusion of the discovery study. For both 5-HTTLPR and STin2 VNTR polymorphisms, there was no significant evidence for an overall effect either in additive, recessive or dominant modeling. The pooled ORs were not excessively affected by the inclusion of any single study. No significant evidence for small-study effects within each group of studies was observed.

Discussion: In contrast to previous meta-analysis which suggested significant associations between the Short allele of the 5-HTTLPR, the STin2.12 allele of the STin2 VNTR polymorphisms and bipolar disorder susceptibility, an updated meta-analysis including more recently published association studies suggests that neither the 5-HTTLPR polymorphism nor the STin2 VNTR polymorphism is likely to be a major determinant of susceptibility to bipolar disorder on a wide population basis.

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109. Neuroimaging Phenotypes for Bipolar Disorder in a Genetically Isolated Population

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Background: Neuroimaging features are particularly promising endophenotypes for psychiatric genetics. Patients with bipolar disorder show structural differences in specific regions of the brain and importantly, for some morphological features, unaffected family members demonstrate intermediate changes compared to controls. We aim to combine the advantages of MRI-based structural brain phenotypes with the power of pedigree-based quantitative genetic methods to identify loci associated with bipolar disorder.

Methods: Familial correlations of total brain, gray and white matter volumes were assessed for images processed to date from members of seven large, multi-generational pedigrees ascertained from two closely related genetically isolated populations, the Central Valley of Costa Rica and Antioquia, Colombia. High resolution brain images were acquired on 1.5T scanners, manually edited to remove non-brain tissue and then segmented to quantify gray and white matter volume. The FCOR procedure in SAGE (Statistical Analysis for Genetic Epidemiology) was used to determine parent-offspring and sib-sib correlations. Data for 44 parent-offspring pairs and 109 sib-sib pairs are included in the current analysis. The threshold for statistical significance was set at $p = 8.3 \times 10^{-3}$ to correct for multiple tests.

Results: Total brain volume and gray matter volume showed significant familial aggregation for both parent-offspring and sibling pairs. The familial correlations for total brain volume were similar for parent-offspring pairs ($r = 0.57$, $p = 7.6 \times 10^{-3}$) and sib-sib pairs ($r = 0.62$, $p = 5 \times 10^{-4}$). Likewise, the correlations for gray matter volume were very similar for parent-offspring pairs ($r = 0.72$, $p = 2 \times 10^{-4}$) and sib-sib pairs ($r = 0.72$, $p = 3 \times 10^{-5}$). White matter volume was significantly correlated among sib-sib pairs ($r = .47$, $p = 7.8 \times 10^{-3}$), and showed a moderate, but not statistically significant correlation within parent-offspring pairs ($r = 0.33$, $p = 0.08$). These correlations agree with the high heritability of brain phenotypes (greater than 0.80 for total brain volume) that have been previously reported among twin pairs.

Discussion: Our preliminary results confirm the high heritability of structural brain phenotypes including total brain volume and gray matter volume. These results are very preliminary, representing approximately 30% of the MRI data that has so far been collected. The larger sample will continue to be collected and analyzed and will improve the statistical power to detect familial aggregation. We anticipate the larger analysis will help in ultimately selecting the most promising brain imaging phenotypes for genome-wide linkage and association analyses.

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110. Shift Work in Nurses: Contribution of Phenotypes and Genotypes to Adaptation

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Background: Daily cycles of sleep/wake, hormones, and physiological processes are often misaligned with behavioral patterns during shift

work, leading to an increased risk of developing cardiovascular/metabolic/gastrointestinal disorders, some types of cancer, and mental disorders including depression and anxiety. It is unclear how chronotype, sleep timing, and circadian clock gene variation contribute to adaptation to shift work.

Methods: Chronotype, newly defined sleep strategies, and genotype for polymorphisms in circadian clock genes were assessed in 388 hospital day- and night-shift nurses.

Results: Generally, early chronotypes ("larks") reported significantly lower self-reported adaptation for night-shift and higher for day-shift, while late chronotypes ("owls") had intermediate adaptation levels for both day and night-shifts. Night-shift nurses who used sleep deprivation in order to switch to day-shift on days-off (~25%) were the most poorly adapted. In addition, polymorphisms in *CLOCK*, *NPAS2*, *PER2*, and *PER3* were significantly associated with outcomes such as alcohol/caffeine consumption and sleepiness, as well as sleep phase, inertia and duration in both single- and multi-locus models. Many of these results were specific to shift type suggesting an interaction between genotype and environment (i.e., shift work).

Discussion: Chronotype, sleep strategy, and genotype are contributors to the ability of the circadian system to adapt to an environment that switches frequently and/or irregularly between different schedules based on the light-dark cycle and social/workplace time. This study of nurses on day- vs. night-shift provides a key example of how an environmental "stress" to the temporal organization of physiology and metabolism can have behavioral and health-related consequences.

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111. Large-Scale Candidate Gene Analysis of Nine Neurophysiological and Neurocognitive Measures Associated with Schizophrenia from the Consortium on the Genetics of Schizophrenia (COGS)

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Background: The exploration of the genetic architecture of neurophysiological and neurocognitive deficits associated with schizophrenia may be a powerful strategy for understanding the genetics of schizophrenia. As part of the Consortium on the Genetics of Schizophrenia (COGS), we have previously created a custom SNP chip consisting of 1,536 SNPs within 94 candidate genes for schizophrenia and related phenotypes, and we have used this chip for the analyses of 12 endophenotypes for schizophrenia. We now report the heritability and association analysis an additional nine secondary measures as candidate endophenotypes for schizophrenia.

Methods: Each of the 130 families previously genotyped for the custom SNP chip consisted of a proband with schizophrenia, both parents, and at least one unaffected sibling. Additional affected and unaffected siblings were included when available for a total of 534 subjects. All subjects were assessed for the following measures: baseline startle reactivity, P50 conditioning amplitude, N100 conditioning amplitude, smooth pursuit eye movements, Degraded-Stimulus Continuous Performance Test (DS-CPT) hit rate, CPT Identical Pairs (CPT-IP) 3-digit d', Letter-Number Span (LNS) immediate recall, and delayed recall and semantic clustering from the California Verbal Learning Test, Second Edition (CVLT-2). A total of 1,385 SNPs remained for analysis following elimination based on quality control thresholds for call rate, cluster separation, and marker informativity.

Results: All nine neurophysiological and neurocognitive measures were found to be significantly heritable in this sample. Association

analyses of these measures have collectively identified associations with 38 genes, many of which were associated with more than one measure. Among these associations was an enrichment of genes related to glutamate signaling. Seven genes displayed evidence for pleiotropy, revealing significant associations with three or more measures, with ERBB4 showing associations to 7 of the 9 measures.

Discussion: The data provide evidence of heritability for nine additional neurophysiological and neurocognitive measures associated with schizophrenia. Association analyses of these measures also provide further evidence to suggest that genes related to the glutamate pathway may play a substantial role in mediating the biological neuropathology of schizophrenia.

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112. Does Depression Kill Your Brain?

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Background: DNA methylation may play a role in the etiology of neuropsychiatric disorders, through abnormal genomic methylation patterns that regulate genes involved in brain development or physiology. In this study we explore the epigenetic profile of major depressive disorder (MDD) throughout the lifespan.

Methods: In order to better understand both the wild type genomic DNA methylation patterns and aberrant methylation events that occur in disease states, we adopted a highly accurate and cost-effective global methylation profiling technique. We used the Illumina Infinium HumanMethylation27 BeadChip (Illumina, Inc.), that targets 27,578 CpG sites across 14,000 genes in the human genome. DNA methylation levels were determined at single CpG resolution within postmortem brain samples from 53 individuals diagnosed with Major Depressive Disorder (MDD) and non-psychiatric controls. All subjects underwent toxicological and neuropathological screens and a psychological autopsy generating DSM Axis I and II diagnoses and medical illness, treatment, and family history. Among the 53 subjects, 25 were MDD cases (15 males and 10 females), and 28 were non-psychiatric controls (19 males and 9 females). The age of the samples spanned a wide range (16 to 89 years) with an average age of 52 and 47 years for MDD cases and controls respectively. We focused our investigation on the dorsal prefrontal cortex due to converging evidence from neuroimaging and functional studies implicating this region in MDD.

Results: In this large-scale study, we interrogated DNA methylation status of a total of 1,461,634 CpGs among all 53 samples. We found that DNA methylation increases throughout the lifespan in human cortex. We identified 477 CpGs showing significant age-related increase in methylation level ($p < 0.05$). Remarkably, the MDD samples show a 3-fold increase in the number of significant CpG sites relative to controls ($p < 2.2 \times 10^{-16}$). Analysis of individual CpG sites comparing methylation patterns in MDD cases vs. controls identified 88 differentially methylated genes ($p < 0.01$). We used these genes in functional pathway analysis and discovered that the only significant functional cluster included genes involved in cell death ($p < 0.01$).

Discussion: Identification of differentially methylated genes involved in cell death is intriguing, and consistent with previous findings

reporting loss of neuronal and glial cells in MDD. These results also suggest that individuals with MDD exhibit greater DNA methylation changes over time, which may significantly contribute to the neuropathology of depression. Supported by MH40210, MH62185, MH64168, K22 HG2915, MH074118

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113. The Impact of a Genome-Wide Supported Psychosis Variant in the ZNF804A Gene on Memory Function in Schizophrenia

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Background: A recent genome-wide association study showed that a variant (rs1344706) in the ZNF804A gene was associated with schizophrenia and bipolar disorder. Replication studies supported the evidence for association between this variant in the ZNF804A gene and schizophrenia and that this variant is the most likely susceptibility variant. Subsequent functional magnetic resonance imaging studies in healthy subjects demonstrated the association of the high-risk ZNF804A variant with neural activation during a memory task and a theory of mind task. As these cognitive performances are disturbed in patients with schizophrenia, this gene may play a role in cognitive dysfunction in schizophrenia. The aim of the current study was to investigate the potential relationship between this ZNF804A polymorphism and memory function.

Methods: The subjects of this study consisted of 113 patients with schizophrenia and 184 healthy control subjects who had been diagnosed according to SCID by a trained psychiatrist. Subjects were excluded from this study if they had neurological or medical conditions that could affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer in an active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. The SNP (rs1344706) was genotyped using the TaqMan 5'-exonuclease allelic discrimination assay. Statistical analyses were performed using SNPAnalyze V5.1.1 Pro software and PASW Statistics 18.0 software. The effects of the high-risk ZNF804A genotype, diagnosis and genotype-diagnosis interaction on verbal memory, visual memory, attention/concentration and delayed recall (measured by the Wechsler Memory Scale-Revised) were analyzed by two-way analysis of covariance.

Results: There was no difference in age, sex, chlorpromazine equivalents of total antipsychotics, age at onset, duration of illness or PANSS scores between genotype groups. The only difference in demographic variables was a significantly greater number of years of education in the control groups. Consistent with previous studies, patients with schizophrenia exhibited poorer performance on all indices as compared to healthy controls ($p < 0.001$). Significant genotype effects were only found for visual memory performance ($p < 0.036$). A significant ZNF804A genotype-diagnosis interaction was also found for visual memory performance ($p = 0.0012$). Patients with the high-risk T/T genotype scored significantly lower on visual memory than G carriers did ($p = 0.018$, effect size: -0.56). In contrast, there was no genotype effect for any index in the controls ($p > 0.05$).

Discussion: Our data suggest that rs1344706 or variations in linkage disequilibrium may be related to memory dysfunction in schizophrenia. We do not know why we found the genotype effect on only Visual memory. A possible explanation is that a previous study reported suggestive linkage evidence for the visual memory on 2q36 near the locus of the ZNF804A gene. Another possibility is that this

SNP is associated with connectivity during N-back memory task, which is an fMRI task using visual cue. This study showed no effect of genotype on a memory task in healthy subjects, which is consistent with our data. A linear genotype effect on connectivity in DLPFC and hippocampal formation during a memory task was found in healthy control subjects in an fMRI study. These data might indicate that quantitative traits (i.e., brain physiological activity measured by fMRI) are closer to the genetic substrate than behavioral traits, such as neuropsychological functions and psychiatric disorders, and should be observable in genetically at-risk but behaviorally unaffected individuals. Such physiological quantitative traits are likely to influence a neuropsychological trait, memory performance, in patients with schizophrenia, however, they might not affect memory performance in healthy subjects. This phenomena suggests that the high-risk SNP in the ZNF804A gene might be related to the neuropsychological disturbance in schizophrenia.

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114. An Analysis of the Interaction Between *MAPK14*, *CNR1* and Marijuana Misuse on White Matter Brain Volume Abnormalities in Schizophrenia

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Background: Longitudinal follow-up of birth cohorts indicates that adolescent marijuana users are at heightened risk for developing schizophrenia compared to non-users. Current theories regarding substance dependence view drug misuse as a means to normalize a fundamental dopaminergic deficit within the reward circuitry. Predisposition for marijuana misuse and for schizophrenia may therefore share common genetic origins. We previously reported that heavy marijuana use in conjunction with specific cannabinoid receptor 1 (*CNR1*) gene variants (rs12720071-G-allele carriers) contributed to greater white matter brain volume deficits and cognitive impairment among schizophrenia probands. In the current study, we investigate the influence of another endocannabinoid-related gene (mitogen-activated protein kinase 14 (*MAPK14*)) on MRI brain morphometry in schizophrenia probands, and its inter-relationships with marijuana misuse and *CNR1*. *MAPK14* encodes a member of the MAP kinases involved in diverse cellular processes, including cannabinoid receptor-induced apoptosis.

Methods: We genotyped 235 schizophrenia patients on 9 tagged SNPs (tSNPs) accounting for a substantial proportion of *MAPK14* genetic variability. Each patient also underwent a high-resolution anatomic brain MRI scan that provided measurements for lobar gray matter (GM) and white matter (WM) volumes. Almost half the sample had no lifetime marijuana use; one-third had marijuana exposure not meeting DSM abuse or dependence criteria, ~15% had marijuana abuse and ~8% marijuana dependence. The effects of single-marker *MAPK14* tSNPs on brain volumes were assessed using ANCOVA (covariates: intracranial volume, gender, age, alcohol/non-marijuana illicit drug use and antipsychotic treatment). A genotype-by-marijuana misuse interaction term was included in the statistical models so as to assess differential *MAPK14* influence on brain volumes across patients with versus without marijuana abuse/dependence. Additional haplotype analyses tested for potential *MAPK14-CNR1* epistasis in conferring brain volume abnormalities among schizophrenia probands with marijuana abuse/dependence.

Results: Among the 9 *MAPK14* tSNPs, only rs12199654 had significant genotype effects on frontal ($p = .003$), temporal ($p = .01$) and parietal

($p = .01$) WM volumes. A-homozygotes had smaller WM volumes than rs12199654-G-allele carriers. There were also statistically significant rs12199654-by-marijuana misuse interaction effects on these lobar WM volumes ($p = .003$, $.03$ and $.01$ respectively). A-homozygotes with marijuana abuse/dependence had significantly smaller WM volumes than the other comparison groups. There were no significant rs12199654 genotype or genotype-by-marijuana misuse effects on GM volumes ($p \geq .08$). Between the four *MAPK14-CNR1* (rs12199654-rs12720071) diplotypes, A-G and G-A haplotypes were significantly associated with WM brain volumes. The A-G haplotype, which comprises *MAPK14* and *CNR1* alleles associated with WM volume deficits, correlated with smaller frontal, temporal and parietal WM volumes ($p \leq .04$). A-G haplotype-by-marijuana misuse interaction effects on frontal and parietal WM approached but did not achieve statistical significance ($p = .08$). G-A haplotype, on the other hand, was related to larger frontal, temporal and parietal WM volumes ($p \leq .009$). G-A haplotype-by-marijuana misuse interaction effects were significant for these WM volumes ($p \leq .02$). Probands with marijuana abuse/dependence and who are rs12199654-G-allele carriers and rs12720071-A-homozygotes had significantly larger frontal, temporal and parietal WM volumes.

Discussion: Given that cannabinoid receptor-induced apoptosis is preceded by increased MAP kinase gene expression, the rs12199654-rs12720071 interactions in this study suggest that epistasis between *MAPK14* and *CNR1* may mediate brain morphometric features of schizophrenia. Our results further indicate that heavy marijuana use in the context of specific *MAPK14-CNR1* haplotypes may contribute to white matter brain volume variations. Schizophrenia and marijuana misuse likely share common genetic predisposition, including mediators of endocannabinoid signaling involved in the homeostasis of dopaminergic tone.

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115. Association of the *NTRK2* Gene with Lithium Response in a Prospective Study and Identification of Candidate Mutations

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Background: Lithium is the original mood stabilizer and the drug with the strongest evidence for efficacy and suicide prevention in bipolar disorder. A subset of patients have a very robust response to lithium with excellent prophylaxis and relief of symptoms. Good lithium responders have specific clinical characteristics such as a strong family history, euphoric mania and good inter-episode recover, and it has been suggested that they are a genetically distinct form of bipolar disorder. Lithium is known to turn on BDNF expression and it is felt its action in part is mediated through the TrkB receptor. We previously reported association of a SNP (rs1387923) in the 3' end of the *NTRK2* gene and lithium response in a retrospectively assessed sample of 184 patients. We now report replication of this association in a pilot analysis of an independent prospective sample.

Methods: 77 veterans were entered into a prospective trial of lithium response. They were first stabilized on lithium over 3-4 months such that they were stable and on monotherapy. After one month of observation, they were then followed for 24 months in order to detect relapse. All 184 subjects were sequenced using dideoxy sequencing across approximately 5 kb under the association peak in the 3' UTR of the gene.

Results: Survival analysis of the total length of treatment showed that the same allele (T) in the same SNP (rs1387923) was also associated with a longer time to relapse ($X^2 = 14.1$, $p = 0.028$). 36 novel variants were identified in the region of association some potentially affecting regulatory regions.

Discussion: These data provide further support for the role of NTRK2 in modulating lithium response and provides some possible functional variants in the gene. This study also demonstrates the advantages and feasibility of using monotherapy and prospective clinical trial methodology in pharmacogenomic studies.

Disclosure: J. Kelsoe: Part 1; Merck, Astra-Zeneca, Psynomics. S. Leckband: None. M. McCarthy: None. A. DeModena: None. T. Shehktman: None.

116. Genome-wide Association Study Of Addiction To Both Licit And Illicit Drugs

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Background: Addiction to both licit and illicit drugs is a common chronic disorder that is extremely costly to the individuals and society. Although genetics contributes significantly to vulnerability to these affective disorders, the susceptibility genes underlying them are largely unknown.

Methods: To identify susceptibility loci and genes for addictions, a case-control-based genome-wide association (GWA) study for each target addiction was conducted on the GENEVA SAGE and NINDS data sets. The phenotypes analyzed included addiction to smoking, measured by Smoking Quantity (SQ), Smoking Urgency (SU), and Fagerström Test for Nicotine Dependence (FTND), and dependence on nicotine, alcohol, marijuana, cocaine, or opiates, which was measured by DSM-IV scales. A total of 3,015 subjects were considered; the number of cases affected by the various addictions differed. We also studied 1,465 controls, who showed no addiction to any licit or illicit drug. Because these DNA samples were genotyped with Illumina 1M bead array or Affymetrix Genomewide 6.0 chip, we performed imputation analyses on those untyped single nucleotide polymorphisms (SNPs) prior to GWA analysis of each addiction with a logistic model.

Results: At the individual SNP level, 523, 528, 513, 520, 559, 473, 515, and 457 SNPs showed association with SQ, SU, FTND, and dependence on nicotine, alcohol, marijuana, cocaine, or opiates, respectively, at a P value of $\leq 5 \times 10^{-4}$. On the basis of the location of each SNP, 179, 178, 153, 167, 168, 167, 166, and 148 genes for these measures were identified. Among them, genes in chromosome 1 open reading frame 107 (C1orf107), contactin 1 (CNTN1), GDP-mannose 4,6-dehydratase (GMDS), and Sp2 transcription factor (SP2) were shared by all addictive measures. For smoking addiction, we identified 70 genes shared by all measures of ND. Further, 13 genes were common to the illicit drug addiction measures marijuana, cocaine, and opiates.

Discussion: Although identification of SNPs and genes associated with specific drug addictions is still in an early stage, the utilization of this GWAS approach for multiple addictions has provided a great opportunity for understanding genetic mechanisms underlying each of these addictive disorders.

Disclosure: S. Yi: None. J. Wang: None. M. Li: Part 1; Li serves as a scientific advisor to ADial Pharmaceuticals.

117. Stress-Induced Dopamine (DA) Release in Cannabis Abuse

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Background: Low striatal dopamine (DA) receptor (D_2) availability and low amphetamine-induced DA release in the ventrostriatal (VST) has been observed in alcoholism, cocaine and heroin dependence. Less is known about the DA transmission in cannabis dependence. A recent study has shown that amphetamine-induced DA release measure with PET/[^{11}C]raclopride was not different between cannabis users (CU) and healthy volunteers (HV). The aim of the present study was to assess with PET/[^{11}C]-(+)-PHNO, $D_{2/3}$ availability in the control

condition and DA release in response to a laboratory stress task (Pruessner 2004) in cannabis abusing individuals.

Methods: 10 medically and psychiatrically healthy cannabis users (CU, 24.8 ± 0.05 years) and 11 healthy volunteers (HV, 25.91 ± 3.9 years) matched for age and sex were included. CU were requested to abstain from using cannabis the day of the PET scan only. Subjects were scanned during a sensorimotor control task (SMCT) and under the stress condition using the validated Montreal Imaging Stress task (MIST). A training session was performed before PET scanning to reduce the effects of novelty. The SMCT and the stress scans were done ~ 7 days apart. The simplified reference tissue method (SRTM) was used to obtain BP_{ND} in each striatal subdivision based on its functional connections to the limbic, frontal executive and motor brain regions: limbic striatum (LST), associative striatum (AST) and sensorimotor striatum (SMST). Stress-induced DA release (indexed as a percent reduction in [^{11}C]-(+)-PHNO BP_{ND}) between CU and HV was tested with ANOVA.

Results: There were no differences in [^{11}C]-(+)-PHNO specific activity or mass across groups, or conditions. SMCT BP_{ND} was significantly different between groups in the AST ($F = 4.98$ $p = 0.03$) with HV having lower BP_{ND} as compared to CU, trend level in the SMST ($F = 3.55$ $p = 0.075$) and not significant in the LST ($F = 2.03$ $p = 0.17$). Percent displacement was not significantly different across groups in any brain region. However a weak trend was found ($F = 1.76$ $p = 0.19$) at the level of the AST, with CU having greater stress-induced changes (CU 1.99%) relative to controls (HV -2.58%). Years of cannabis use was significantly associated with full-striatum displacement ($r = 0.69$ $p = 0.04$), and trend level in the LST ($r = 0.53$ $p = 0.14$), with no effect in any other brain region.

Discussion: SMCT BP_{ND} was lower as expected from previous studies, however our preliminary results do not seem to suggest a blunted DA release in cannabis users. Consistent with Urban study, we observed alterations in the AST rather than VST. Dysregulation in this area, may indicate milder but topographically different dopaminergic involvement in CU as opposed to other drugs, but similar to the alterations reported in psychosis.

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118. Kynurenines in Bipolar Disorder: State Marker or Trait Marker?

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Background: Kynurenine pathway disturbances in the serum of drug naïve or drug free patients with bipolar mania on admission and after 6 weeks treatment with mood stabilizers had been reported (Myint et al, 2007). However, a question is raised as to whether these kynurenines be state or trait marker.

Methods: We have carried out two studies. First, we investigated the kynurenine changes in the serum of 60 patients with bipolar disorders of different states. Second, the SNPs in tryptophan hydroxylase II, kynurenine monooxygenase and kynurenine aminotransferase III (KAT III) enzymes of 72 patients with bipolar depression and 72 age and gender matched normal controls were studied. The associations between SNPs, haplotypes and diagnosis or specific symptoms were analysed.

Results: All 3 important markers; serum kynurenine acid, kynurenine acid/kynurenine ratio and kynurenine acid/3-hydroxykynurenine ratio showed significant decreases in symptomatic states than in euthymic state. The KAT III gene polymorphism showed an association with bipolar disorder. Particularly, the haplotype containing the particular polymorphism were associated with depressive symptoms in combination with anxiety symptoms.

Discussion: The findings demonstrated that the kynurenine markers can be the state markers in bipolar disorders. However, these changes

may also be partially due to the KAT III gene polymorphism. Further studies on gene-biochemical interaction should be carried out.

Disclosure: **A. Myint:** *Part 1;* I am a consultant at a diagnostic company, Advanced Practical Diagnostics n.v. in Belgium. *Part 2;* 50% of my income is paid by Advanced Practical Diagnostics n.v., Belgium. *Part 3;* I am the first inventor in patents where the Advanced Practical Diagnostics n. v. is one of the applicants together with the university. *Part 4;* The research study is funded by European Commission. *Part 5;* I am paid 50% by the company Advanced Practical Diagnostics n.v., Belgium and 50% paid by European project. **M. Rothermundt:** *Part 3;* Prof. Rothermundt is one of the inventors in a filed patent in which Advanced Practical Diagnostics is one of the applicants together with the university. **M. Schwarz:** Dr. Schwarz is one of the inventors in a granted patent where Advanced Practical Diagnostics is one of the applicant together with the university. **M. Muelbacher:** None. **S. Claes:** *Part 4;* Prof. Claes is one of the inventors in a filed patent where Advanced Practical Diagnostics is one of the applicant together with the universities.

119. WNT Signaling in Depression and Suicide: Postmortem Brain Studies

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Background: GSK-3 β and beta-catenin are important components of the WNT signaling pathway. In this signaling pathway, WNT interacts with the frizzled family of receptors leading to the phosphorylation of dishevelled, which in turn activates GSK-3 β , leading to the stabilization and accumulation of beta-catenin. GSK-3 β is a serine/threonine kinase and has been implicated in the hormonal control of several regulatory proteins. beta-Catenin, whose activity is regulated by GSK-3 β is an important transcription factor involved in the transcription of many genes. There is some evidence which suggests that both GSK-3 β and beta-catenin maybe involved in the pathophysiology of mood disorders. It has also been shown that GSK-3 β may be a target for the action of Lithium, a mood stabilizing drug that causes inhibition of GSK-3 β activity and whose neurotrophic and beneficial effects maybe related to its effects of on GSK-3 β . Since Lithium is also a drug in the treatment of suicidal behavior, it is quite possible that alterations in GSK-3 β and possibly beta-catenin maybe associated with the pathophysiology of suicide. We have therefore studied the role of WNT signaling pathway in depression and suicide by determining the protein expression of GSK-3 β and beta-catenin in various areas of the postmortem brain obtained from depressed suicide victims, non-suicidal depressed subjects and normal control subjects.

Methods: Postmortem brain samples were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland. Tissues were collected only after a family member gave informed content. The psychiatric diagnosis was based on structured clinical interview for DSM-IV (SCID) and was based on interviewing at least one family member and a friend of the studied subjects. The protein expression of GSK-3 β and beta-catenin was determined using the western blot technique. The protein expression of GSK-3 β and beta-catenin was determined in the membrane and in the cytosolic fractions in the PFC obtained from 27 normal control subjects, 23 depressed suicide subjects and 12 non-suicidal depressed subjects. GSK-3 β protein expression in cytosolic and membrane fractions was determined in the hippocampus obtained from 23 normal control subjects, 24 depressed suicide subjects and 7 non-suicidal depressed subjects.

Results: We observed a significant decrease in the protein expression levels of GSK-3 β in both cytosolic and membrane fractions of PFC and hippocampus in suicidal and non-suicidal depressed subjects as compared to normal control subjects. Protein expression of

beta-catenin was also significantly decreased in the cytosolic and membrane fractions of PFC from non.

Discussion: These studies suggest that abnormalities of WNT signaling pathway may play an important role in the pathophysiology of depression and that this abnormality in WNT signaling pathway in depression may be related to decreased expression of GSK-3 β and beta-catenin in depression. (Supported by NIMH RO1 48153 and Distinguished Investigators grant from American Foundation for Suicide Prevention.)

Disclosure: **G. Pandey:** None. **X. Ren:** None. **H. S. Rizavi:** None. **R. Roberts:** None. **Y. Dwivedi:** None.

120. Discovery of Protein Biomarkers in Cerebrospinal Fluid (CSF) from Patients with Civilian Post Traumatic Stress Disorder (PTSD).

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Background: Post Traumatic Stress Disorder (PTSD) affects 7-8% of the general population of the United States and approximately 15% of veterans returning from combat. The symptoms can persist for months or decades, and the diagnosis is presently based solely on the patient's history and behavioral symptoms. Unfortunately we do not fully understand the biological mechanisms underlying PTSD. Therefore, attempts at drug discovery for this disorder are severely compromised. However, we have hypothesized that cerebrospinal fluid might contain proteins that could yield hints as to the mechanistic basis for PTSD.

Methods: Fourteen medication-free outpatients with chronic civilian PTSD (34.9 ± 10.4 years old, 10 women) and ten non-traumatized, healthy subjects (35.3 ± 13.1 years old, 7 women) participated in the study. Traumas were prepubertal in 5 subjects and adults in nine. Time elapsed from trauma exposure was 26 ± 4 years in pre-pubertal trauma, and 10.1 ± 8.8 years in adult exposure. Patients were physically healthy, with no psychotropic medication for at least three weeks prior to lumbar puncture (or six weeks for patients on fluoxetine), and did not meet criteria for alcohol or substance abuse, or dependence, for at least six months prior to the study. Psychiatric diagnoses were established using the Structured Clinical Interview for DSM-IV (SCID), and the severity of PTSD was determined using the Clinician-Administered PTSD Scale (CAPS). Severity of depressive, anxiety and overall symptoms was assessed using the Inventory of Depressive Symptomatology (IDS), Hamilton Anxiety Rating Scale (HAMA) and Clinical Global Impression - Severity scale (CGI-S), respectively. Individuals with PTSD and controls did not differ with regard to age, gender distribution, race, or body mass index (BMI). Severity of PTSD was moderate, with a CAPS score of 73.1 ± 10.3 . Depression (IDS 16.4 ± 8.2), Anxiety (HAMA 13.1 ± 6.8) and overall symptom severity levels (CGI-S 4 ± 1.2) were moderate as well. Lumbar Puncture (LP) was performed between 8:00 and 9:00 AM by an experienced physician. A 20-gauge introducer needle was inserted and approximately 15 cc of CSF was withdrawn and frozen in aliquots at -80°C for later assay. Proteins in CSF were labeled with the Cy3 fluorescent dye, and a sample of a standard serum was labeled with Cy5 to provide a common standard across all experiments. The mixture was incubated with a 507-duplicate feature antibody microarray, and imaged on a Perkin-Elmer ScanArray2 fluorescence slide reader. Samples of CSF were also analyzed on a Reverse Capture Protein Microarray platform. Significance was based on t-tests ($p < 0.05$) and a local False Discovery Rate of $< 10\%$.

Results: We have identified ten proteins which significantly distinguish between CSF from PTSD patients and Healthy Controls, independently of gender. Among the top proteins we find (i) synaptotagmin [$p = 6 \text{ EXP}(-9)$], a protein associated with calcium dependent neurotransmitter exocytosis; (ii) Ubiquitin E3 Ligase [$p = 2 \text{ EXP}(-7)$], a protein in which mutations are associated with forms of mental retardation; and (iii), the Small Inducible Cytokine

Subfamily E, member 1 (SCYE1/EMAPII; [$p = 2 \times 10^{-6}$]), a protein chemoattractant for microglia, and found to be elevated in spinal cord injury. In addition, we have identified different sets of proteins which significantly discriminate between CSFs from Male and Female PTSD patients. CSF from Male PTSD patients is significantly characterized by many proteins associated with proinflammatory signaling pathways. Importantly, these proteins do not distinguish between Male and Female Healthy Controls.

Discussion: The differential proteomic signatures in CSF from PTSD and control patients suggest that PTSD is associated with changes in the biology of the Central Nervous System. The fact that male and female PTSD patients differ in proteomic signature is not a clinical surprise and lends credibility to this approach in PTSD research. Still, the small number of male subjects in both patient and control groups calls for larger and better balanced replication studies. In conclusion, we believe that the insights revealed by the proteomic approach may have consequences for understanding the biological mechanism(s) underlying PTSD, and may provide clues for the discovery and development of novel PTSD therapeutic agents. Support: DOD, CDMRP (PI: HBP); DOD, CNRM (PI: HBP); NIMH intramural 02-M-0317 (PI: OB).

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121. Cortical Mu Opioid Receptor mRNA Expression in Schizophrenia and Across Development

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Background: Prefrontal cortical (PFC) dysfunction in schizophrenia has been linked to disturbances in GABA neurons, including parvalbumin- and somatostatin-containing cells. Interestingly, the endogenous opioid system modulates parvalbumin and somatostatin neuron function in a manner that could directly impact GABA-related disturbances in schizophrenia. For example, activation of the mu opioid receptor (MOR) suppresses both the activity of, and GABA release from, parvalbumin and somatostatin neurons in rat brain. Thus, alterations in PFC MOR signaling, especially if they arise during development, could contribute to cell type-specific disturbances of GABA neurotransmission in schizophrenia. Consequently, in this study we sought to determine if 1) MOR mRNA levels are altered in the PFC in schizophrenia; 2) other opioid markers, including the δ opioid receptor (DOR) and the opioid ligand propeptide proenkephalin, are similarly altered in schizophrenia; 3) antipsychotic medications or other factors commonly associated with schizophrenia affect MOR mRNA expression; 4) MOR mRNA levels are related to predictors or measures of disease severity; and 5) MOR mRNA expression has a distinctive developmental trajectory in the PFC of monkeys.

Methods: We used quantitative PCR (qPCR) to measure mRNA levels for MOR and other opioid markers, including DOR and proenkephalin, in PFC area 9 from 42 schizophrenia subjects, each matched to one healthy comparison subject for sex and age. The mean age, postmortem interval, brain pH, RNA integrity number, and tissue storage time did not differ between subject groups. qPCR was performed using the comparative threshold cycle method with four replicate measures per target gene, and target gene expression levels were normalized using three reference genes. All primer pairs demonstrated high amplification efficacy ($>96\%$). An analysis of covariance model was used to test the effect of diagnosis on relative expression level for each target mRNA with storage time, brain pH, and RNA integrity number as covariates, and subject pair as a blocking factor. We also conducted similar studies in the PFC of monkeys chronically exposed to haloperidol, olanzapine, or placebo ($n = 6$ per

treatment condition) for at least 17 months and of a developmental series of 49 monkeys ranging in age from 1 week to 11.5 years.

Results: We found higher mRNA levels for MOR ($+27\%$), but no differences in DOR or proenkephalin mRNAs, in schizophrenia subjects relative to healthy comparison subjects, and these results were confirmed using a second primer set designed against a different region of each transcript. Elevated PFC MOR mRNA levels in schizophrenia appeared to be predominantly attributable to higher mRNA levels of the exon 4-containing MOR-1 splice variant and not other splice variants. Higher MOR mRNA levels in schizophrenia also appeared to be related to some predictors and measures of disease severity, but were not a consequence of exposure to substances of abuse, psychotropic medications, or other potential confounds. Finally, MOR mRNA levels markedly declined through early development, stabilized shortly before adolescence, and substantially increased with age across adulthood in monkey PFC.

Discussion: The combination of higher MOR mRNA levels in schizophrenia and a normal decline in MOR mRNA levels prior to adolescence suggests that a developmental pause or impeded maturation of PFC MOR mRNA expression may occur in the disorder. Higher MOR mRNA levels, if accompanied by a corresponding increase in protein levels, may lead to suppression of GABA release from, and somatic hyperpolarization of, parvalbumin and somatostatin neurons, which together may impair the functioning of these neurons in schizophrenia.

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122. Reduced serotonergic neurotransmission and lapses of attention in children and adolescents with attention deficit hyperactivity disorder

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Background: Changed serotonergic (5-HT) neurotransmission has been linked to altered attention and memory processes. Attention deficit hyperactivity disorder (ADHD) was shown to be associated with impaired attention and working memory. However, studies on serotonergic functioning involving children and adolescents with ADHD are scarce. The present study investigated the effects of a diminished 5-HT turnover achieved by rapid tryptophan depletion (RTD) on attentional performance in children and adolescents with ADHD.

Methods: A sample of twenty-two male patients with ADHD (aged 9-15 yr) enrolled in the study. All patients received the RTD procedure Moja-De and a tryptophan (Trp)-balanced placebo in a randomized, double-blind, within-subject crossover design on two separate study days. Lapses of attention and phasic alertness were assessed within the test battery for attentional performance under depleted and sham-depleted conditions at three time-points: 120 (T1), 220 (T2) and 300 (T3) minutes after intake of RTD/Placebo.

Results: At T1 there was a significant main effect for RTD, indicating more lapses of attention under intake of a Trp-balanced placebo compared to diminished 5-HT neurotransmission. For the time-points T2 and T3 there were no such effects. Phasic alertness was not affected by the factors RTD/Placebo and time.

Discussion: The results of the present study are in line with findings in adults suggesting that changed availability of the 5-HT precursor Trp influences gating functions in the human brain, which in turn can affect attentional performance. Interactions of 5-HT with other neurotransmitters as possible underlying neurochemical processes

could be subject to further investigations involving healthy controls as regards altered attentional performance in children and adolescents.

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123. Increased Striatal Activation in Compulsive Indoor Tanners upon Exposure to Ultraviolet Light Compared to Sham Light

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Background: Many tanners exhibit behaviors consistent with an addictive disorder. The neural mechanisms underlying compulsive tanning has not been explored. This study was designed to assess changes in central nervous system functioning induced by ultraviolet radiation (active UVR) vs. sham UVR (UVR filtered) in subjects who met criteria consistent with an addictive tanning disorder.

Methods: Seven [4 women; 30.7 ± 9.0 years old (mean \pm SD)] indoor tanners were studied. Subjects were placed under a UVA/UVB emitting tanning light during two sessions. Two visually identical plastic/acrylic filters were placed over the tanning light. Filters were transparent to visible/infrared light and heat; one blocked UVR and one did not. Session order was randomized and subjects were blinded to study order. Regional cerebral blood flow (rCBF) during active and sham UVR sessions was assessed using single photon emission tomography (SPECT). The radioligand was administered immediately upon activation of the tanning light.

Results: SPECT imaging revealed increased ($p < 0.01$) rCBF in the dorsal striatum, including both the caudate and putamen, during active UVR exposure compared to sham UVR. When exposed to active UVR, relative to sham UVR, subjects reported a decrease in desire to tan ($p < 0.002$). Of the six subjects expressing a preference for a tanning bed, five chose the active UVR condition.

Discussion: These preliminary findings offer a novel methodology to assess the neural response to rewarding stimuli and suggest that UVR may have centrally rewarding properties that encourage excessive tanning.

Disclosure: B. Adinoff: *Part 1*; University of New Mexico, Medical University of South Carolina, American Inst of Biological Sciences, American Academy of Addiction Psychiatry, Methodist Medical Center (Dallas, TX), Vanderbilt University, University of North Texas Health Care System, John Peter Smith Hospital, Ft. Worth, Shook, Hardy & Bacon LLP representing tobacco companies, Paul J. Passante, P.C. (medical malpractice consultant). C. Harrington: None. M. Devous: Scientific Advisory Board of AVID Radiopharmaceuticals. *Part 4*; AVID Radiopharmaceuticals, Alseres. H. Jacobe: None. T. Harris: None.

124. Prefrontal N-acetylaspartate Concentrations in Manic Bipolar Patients Associated with Treatment Response to Quetiapine

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Background: Several lines of evidence suggest that affective symptoms in patients with bipolar disorder may be related to functional abnormalities in the anterior limbic network in general, and in prefrontal regions in particular. Increased ventrolateral prefrontal cortex (VLPFC) activity observed in euthymic bipolar patients gives way to decrements in functional activation during mania, suggesting a loss of modulatory control over limbic structures involved in emotional expression with which the VLPFC is closely networked, including portions of the anterior cingulate cortex (ACC) and

amygdala. *Pari passu* with these findings, evidence of decreased neuronal metabolism is reflected in magnetic resonance spectroscopy (MRS) findings including prefrontal n-acetylaspartate (NAA) changes. **Methods:** Twenty manic bipolar patients (seven women, age: 29 ± 8 years) participated in baseline MRS scans contemporaneously with starting eight week trials of quetiapine. Bipolar mania was diagnosed using the structured clinical interview for DSM-IV, and a Young mania rating scale (YMRS) score of twenty or greater. Eleven patients (four women, age: 29 ± 7) remitted, defined as a YMRS score less than 13, and nine patients remained symptomatic (three women, age: 29 ± 10). Baseline YMRS did not differ between groups ($p = 0.29$). Quetiapine was dosed according to our usual clinical practice. MRS data was available at endpoint for nineteen patients (seven women, age: 28 ± 8), ten of whom (six women) were found to have remitted. Twelve healthy subjects (seven women, age: 30 ± 7 years) also participated in MRS scans; eight week follow-up MRS data was available for seven (four women) of these healthy subjects. MRS data were acquired for three, eight cm³ voxels centered on the left and right ventrolateral prefrontal cortex (L & RVL PFC) and ACC using short-echo single-voxel PRESS pulse sequences on a 4T Varian MRI scanner. Spectra were processed, and quantitative NAA values obtained, using LCModel software.

Results: NAA values in bipolar patients did not differ from those of healthy subjects at baseline in the LVPFC, RVL PFC, or ACC. Bipolar patients who ultimately responded to quetiapine treatment however, showed increased baseline concentrations of NAA in the RVL PFC ($p = 0.04$, Cohen's $d = 1.01$) and decreased baseline concentrations in the ACC ($p = 0.07$, Cohen's $d = 0.97$), compared with patients who failed to respond. Following treatment, concentrations of NAA in the ACC significantly increased in patients who responded to quetiapine, compared with patients who did not respond to treatment ($p = 0.04$, Cohen's $d = 1.26$), rising over the eight weeks in the former and falling slightly in the latter. NAA changes in neither the RVL PFC ($p = 0.99$) nor LVPFC ($p = 0.52$) differed between patients who remitted and those who did not. NAA changes did not significantly differ between healthy subjects and remitting patients, non-remitting patients, or bipolar patients as a whole.

Discussion: NAA is a complex neurochemical marker that may be used to model both neuronal integrity and metabolism. While disentangling the contributions of each to baseline NAA values may be problematic, short term changes are presumably more likely to reflect effects on neuronal metabolism rather than rapid change in neuronal number. Our findings suggest that bipolar manic patients who will respond to quetiapine have greater baseline RVL PFC neuronal integrity and/or fewer metabolic deficits in this region, compared with patients who are less likely to remit. Conversely, NAA values are relatively low in the ACC of patients who go on to remit over eight weeks of treatment, compared with non-remitters. Previous studies have suggested the appearance of progressive prefrontal changes in bipolar disorder; these NAA differences may reflect different stages of the underlying illness. More speculatively however, differences in baseline NAA may be markers of neurofunctionally distinct patient subsets. With treatment, remitting patients show an increase in ACC NAA, while non-remitters actually show a non-significant decrease - suggesting recovery of ACC function, either as a driver of treatment response or secondarily to the resolution of manic symptoms. Although the small number of participants makes identification of these findings as biomarkers for quetiapine response quite preliminary, the high effect sizes observed here suggest that MRS may ultimately play a role in predictive models of treatment response in bipolar mania. Ultimately, larger studies examining a wider range of therapeutic interventions will be necessary to confirm and expand these findings.

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125. Altered Cortical Network Topologies Associated with Type 2 Diabetes and Major Depression

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Background: Previous work from our group has shown that subjects with diabetes and depression have gray matter volume decreases, cortical thinning, and deficits in attention and executive function compared to healthy control subjects and subjects with diabetes alone. The purpose of this study was to examine cortical network properties in subjects with diabetes and diabetes with depression compared to healthy control subjects.

Methods: Subjects were scanned with a 1.5T GE scanner. Freesurfer (<http://surfer.nmr.mgh.harvard.edu>) was used to generate cortical thickness measurements using an automatic parcellation method. Interregional cortical thickness correlations were measured after controlling for age, gender, and overall cortical thickness. These correlations were used to generate a binary undirected network representing cortical connectivity. Network metrics such as clustering coefficient, shortest path length, global and local efficiency, and sigma were calculated for all three groups using the Brain Connectivity Toolbox (<http://www.brain-connectivity-toolbox.net>).

Results: Diabetic depressed subjects had marked hemispheric differences in correlation patterns compared to healthy control subjects and diabetic subjects. All three subject groups demonstrated small-world network properties characterized by $\sigma > 1$ but depressed diabetic subjects had higher clustering coefficients and longer average path lengths compared to healthy controls and diabetic subjects. Depressed diabetic subjects had lower global efficiencies compared to the other comparison groups.

Discussion: This preliminary study suggests that cortical networks are impaired in patients with diabetes and depression. Further work will be done to characterize specific disconnection patterns which may be correlated with cognitive function and mood.

Disclosure: O. Ajilore: None. A. Kumar: None.

126. Pharmacologically-controlled Changes in Gonadal Steroid Hormones Influence Gray Matter Volume in the Orbitofrontal Cortex and Hippocampal Regions of Women

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Background: Preclinical studies in animals document that the gonadal steroid hormones, estrogen and progesterone, modulate neuronal activity, brain morphology, and behavior. However, while several neurofunctional studies of hormonal effects in humans have been published (Berman et al. 1997; Dreher et al. 2007; Protopopescu et al. 2005), there is little conclusive evidence of effects on human brain morphology. Hippocampal gray matter (GM) volume has been reported to vary across the menstrual cycle, with increased GM during the follicular phase, compared to the luteal phase (Protopopescu et al. 2008), but the effects of estrogen and progesterone, independent of each other, on the GM volume of the human brain have not been investigated. To address this open question, we analyzed structural MRIs obtained during three phases of a hormonal manipulation protocol that pharmacologically produces temporary menopause and adds back progesterone and estrogen separately.

Methods: Structural MRI scans were obtained from 17 healthy female volunteers (mean age 33 ± 6 years) with no history of psychiatric or medical illness. Data were collected during each of three hormone conditions: (1) ovarian suppression (i.e. temporary menopause)

induced by the gonadotropin-releasing hormone agonist leuprolide acetate (LUP), (2) Lupron plus estrogen replacement (EST), and (3) Lupron plus progesterone replacement (PROG). The order of the progesterone and estrogen conditions was randomized and counter-balanced across participants. Using 3 Tesla MRI scanners, we collected axial slices with voxel sizes of $0.9375 \times 0.9375 \times 1.2$ mm using a three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRage) sequence. Scanner and scanning parameters were kept constant during all three conditions for every subject, and a scan was acquired during each condition. GM volume analyses were performed using standard Voxel-Based Morphometry (VBM) with diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) normalization (Ashburner 2007). An analysis of covariance with intracranial volume and scanner type entered as covariates was performed using SPM5. Pairwise comparisons between hormone conditions are reported at $p < 0.005$, whole brain, uncorrected.

Results: GM volume in bilateral hippocampal regions increased during PROG compared to EST and LUP conditions. GM volume also increased in EST compared to PROG treatment in the left insula. Finally, left lateral orbitofrontal cortex (lOFC) GM volume increased in EST compared to PROG treatment, while the left medial OFC (mOFC) showed increased GM volume in PROG relative to EST treatment.

Discussion: Our data suggest that while the exact neurobiological meaning of these findings remains to be elucidated, gonadal steroids affect brain morphology as measured with MRI. Our findings also indicate that the gonadal steroid hormones, estrogen and progesterone, affect morphology in areas of the brain associated with the limbic system and reward processing. In contrast to previous literature, we found a GM volume increase in the hippocampal region with progesterone, a change usually associated with estrogen (Protopopescu et al. 2008; Woolley et al. 1992). However, studies across the menstrual cycle do not disentangle the effects of estrogen and progesterone, and the estrogen treatment-related changes in neuronal microstructure reported in animals (Woolley et al. 1992) may not correspond to VBM measurements, where the results could reflect not only alterations in neuropil, but also vascular modulation or altered water content. The insula, where we saw increased GM volume during estrogen treatment, shares bidirectional cortical projections with the OFC (Reynolds et al. 2005) and has been implicated in reward processing, specifically error prediction (Preuschoff et al. 2008). Our opposite findings in lOFC vs. mOFC, with increased GM volume with estrogen vs. progesterone, respectively, are consistent with known functional distinctions between these two OFC subdivisions (Elliot et al. 2000; Ongur et al. 2000). Further research is needed to elucidate how these changes in GM volume relate to changes in behavior and neural function.

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127. Subgenual resting blood flow accurately discriminate depressed individuals as bipolar I versus unipolar using arterial spin labeling pattern recognition analysis

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Background: Distinguishing bipolar depression from unipolar depression is a major clinical challenge. Abnormal anterior cingulate cortex

metabolism and blood flow are reported in unipolar depression. Whether these measures can distinguish unipolar from bipolar depression remains unexamined. In this study, we first determined whether arterial spin labeling (ASL), a novel non-invasive neuroimaging technique measuring blood flow, could identify abnormal anterior cingulate cortical blood flow in unipolar depression; and second, whether pattern recognition analysis using ASL-measured anterior cingulate cortical blood flow could accurately classify individuals with unipolar versus bipolar depression.

Method: We examined 1) anterior cingulate cortical blood flow with ASL and gray matter volume in eighteen recurrent depressed individuals (DSM-IV-TR criteria) versus eighteen age-matched healthy control participants. 2) We used pattern recognition analysis with support vector machine learning, and leave-one-out cross validation, to classify cerebral blood flow in eleven independent recurrent unipolar depressed, and eleven bipolar type I depressed, individuals in each anterior cingulate cortical subdivision (BA32, 24 and 25).

Results: 1. The eighteen unipolar depressed individuals showed significantly greater anterior cingulate cortical blood flow in BA24/32 subdivisions ($p = 0.002$), but no abnormalities in anterior cingulate cortical gray matter volume, versus eighteen healthy controls. 2. Pattern recognition analysis of the subgenual anterior cingulate cortical blood flow classified individuals with unipolar versus bipolar depression at 82% accuracy ($p = 0.001$). Rostral anterior cingulate cortical blood flow classified individuals with unipolar versus bipolar depression at 63.6 % accuracy (BA32; $p = 0.1$) and 68.2% accuracy (BA24; $p = 0.028$). Medication had no impact on the classification analysis.

Discussion: This is the first study to use ASL, a promising and non-invasive neuroimaging technique, to identify the extent to which measures of abnormal resting cerebral blood flow in recurrent unipolar depression could have clinical utility as measures to distinguish recurrent unipolar from bipolar depression. We first showed that abnormally elevated anterior cingulate cortical blood flow in unipolar depression when compared to healthy individuals. We further show that the ASL measure of resting blood flow in the subgenual subdivision of the anterior cingulate cortex (BA25) classified, case by case, a second, independent group of unipolar depressed individuals and a group of bipolar type I depressed individuals with high sensitivity, high specificity and 82% accuracy. These novel neuroimaging methodologies, based upon biological measures that reflect pathophysiologic abnormalities, can potentially help to classify individuals into psychiatric diagnostic groups.

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128. Resting State Connectivity Differentiation Between Unipolar and Bipolar Depression

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Background: It is critical to be able to differentiate between unipolar depression (UD) and bipolar disorder depression (BDD) both for understanding the etiology of these similar but at the same time very different illnesses as well as for determining the type of treatment (mood stabilizers alone, antidepressants alone or a combination) that is indicated. In this study, we investigated differences between the UD and BDD using fMRI resting state cortico-amygdalar connectivity.

Method: Unmedicated UD and BDD patients and matched healthy controls underwent functional magnetic resonance imaging (fMRI) imaging. In each session, a resting state connectivity scan was obtained. Using left amygdala (IAMYG) as the seed region, correlations of low frequency BOLD fluctuations (LFBF) with all other voxels of the brain were calculated.

Results: In the ongoing study we have analyzed data from 13 BDD (Age: 32 ± 9 ; 8F), 26 UD subjects (Age: 32 ± 9 ; 16F), and 13 matched

healthy subjects (Age: 30 ± 9 ; 8F). UD subjects had decreased left amygdalar-ACC connectivity compared to healthy controls ($p < 0.05$) while BDD subjects did not exhibit this difference. BDD subjects had increased left amygdalar-vACC connectivity compared to UD subjects ($p < 0.05$).

Conclusion: ACC-left amygdalar resting state connectivity differences could serve as a potential biomarker for differentiating between UD and BDD. Further study is needed.

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129. Hippocampal Volume Differences in Gulf War Veterans with Current versus Lifetime PTSD Symptoms

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Background: Magnetic resonance imaging (MRI) studies have implicated that PTSD is associated with a reduced size of the hippocampus. However, the nature of this relation is poorly understood. Two fundamentally different ideas are currently considered. According to one, largely supported by animal studies, a small hippocampus is considered a sequel of chronic stress reactivity in PTSD. According to the other, supported by a PTSD imaging study of homozygous twins discordant for trauma exposure, a pre-existing small hippocampus increases the vulnerability to develop PTSD. The relation is even more complex because PTSD is often comorbid with depression which is also thought to be associated with smaller hippocampal volume. We sought to test the hypothesis that veterans with remitted as well as current PTSD would have smaller hippocampal volumes compared to veterans without PTSD, independent of the effects of depression.

Methods: Clinical and MRI data were collected in a cross sectional study of 244 Gulf War veterans. Measures included lifetime and current CAPS, HAM-D, Life Stressor Checklist and Lifetime Drinking History. MRI data were acquired with a 1.5 Tesla scanner. Hippocampus was measured using a commercially available brain mapping tool (Medtronic Surgical Navigation Technologies, Louisville, CO) by first, placing manually 22 control points as local landmarks for the hippocampus on individual brain MRI data and second, by applying fluid image transformations to match the individual brains to a template brain. The pixels corresponding to the hippocampus were then labeled and counted to obtain volumes. Intracranial volume (ICV) was measured using FreeSurfer, a software that uses an atlas based spatial normalization procedure on T1 weighted images. The main statistical analysis was a hierarchical regression with hippocampal volume adjusted for ICV as outcome variable. Demographic factors, trauma experience, substance use, antidepressant treatment and clinical symptom scores were entered as independent predictors. To determine if hippocampal volumes differed among participants with chronic PTSD, those with remitted PTSD, and those who never developed PTSD, a one-way ANOVA was performed. The statistic package SPSS 16.0 (SPSS Inc., Chicago, IL) was used.

Results: We had a predominantly male Caucasian population with a mean age of 45. Defining PTSD as a CAPS score of ≥ 40 and depression as a HAM-D score of ≥ 14 , 82 veterans had lifetime PTSD, 44 current PTSD and 38 current depression. In the linear regression analysis current PTSD symptoms (standardized coefficient $\beta = -0.25$, $p = 0.03$), but neither lifetime PTSD symptoms nor current depression were associated with smaller hippocampal volume. Gender, age, history of early life trauma, education, current or lifetime alcohol use, current marijuana use and antidepressants did not have independent effects. Participants with chronic PTSD had on average a smaller hippocampus compared with those with remitted PTSD (mean difference 6.5%, $p < 0.05$) or those who never developed PTSD (mean difference 5.1%, $p < 0.05$).

Discussion: The finding that current but not lifetime PTSD symptom severity explains hippocampal size raises two possibilities: either a small hippocampus is a risk factor for lack of recovery from PTSD (trait), or PTSD effects on hippocampal volume are reversible once the patient recovers (state). The trait hypothesis modifies the conclusion of Gilbertson's twin study of smaller hippocampal volume being a familial risk factor for PTSD. That study had excluded individuals who had recovered from PTSD. The state hypothesis is supported by other studies showing that hippocampal size was negatively correlated with duration and severity of PTSD symptoms and increased after long-term paroxetine therapy in PTSD patients. A growing literature in other diseases shows that the hippocampus can change in response to exercise, pharmacologic interventions and alcohol abstinence. The state hypothesis is also supported by our previous finding in PTSD patients of reduced volume in the hippocampal subfield CA3 and the dentate gyrus, areas which are known to undergo neurogenesis in adulthood. As our data are from a cross-sectional study with measurement of hippocampal volume at one time point only we cannot determine which of these two interpretations is correct.

Other limitations are dependence on subjective reports and a study population which may lack generalizability. However, our data in more than 200 well characterized participants suggest that presence of chronic PTSD symptoms rather than remitted PTSD is associated with a smaller hippocampus.

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130. Unique influence of PTSD symptom clusters on neural activations during anticipatory processing

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Background: Intimate partner violence (IPV) is one of the most common causes of posttraumatic stress disorder (PTSD) in women. Anticipatory anxiety is thought to contribute to avoidance behavior, is often associated with PTSD, and may play a role in the maintenance of the disorder. Previous research with IPV-related PTSD has reported increased insula activation and decreased functional connectivity between insula and amygdala during anticipation of negative stimuli. Recent PTSD research involving symptom provocation (e.g., processing of trauma scripts) suggests the type of emotional responses (e.g., reexperiencing vs. dissociative) may relate to unique neural activation patterns. However, no study has yet examined whether separate PTSD symptom clusters relate to unique neural activation patterns during anticipatory processing.

Methods: The current study has enrolled 32 PTSD and 29 healthy control (HC) participants who completed clinical measures and an fMRI anticipation paradigm. The Clinician-Administered PTSD Scale (CAPS) was used to diagnose PTSD as well as to characterize the severity level of PTSD (total mean score = 68.07), including the re-experiencing, hyperarousal, and avoidance clusters. Groups did not differ in regards to age, but HCs had higher levels of education than PTSD subjects. A BOLD functional magnetic resonance imaging (fMRI) scan was completed during the anticipation task, which consisted of a continuous performance task interspersed with cued presentation of positive and negative affective stimuli. The primary regressors of interest include the anticipation periods for both positive (API) and negative images (ANI). Primary regions of interest (ROIs) included the anterior insula, amygdala, and medial prefrontal cortex (mPFC). ROI analysis was performed using linear mixed models to examine the interaction effects of group (PTSD vs. HC) and valence (ANI vs. API) while covarying for education level. Within the PTSD group, Huber robust partial regressions were performed to examine

the unique influence of PTSD symptom clusters on neural activations for the ANI-API contrast.

Results: Valence effects were evident within the bilateral anterior insula, which were greater for ANI than API. However, regions of the bilateral amygdala and medial PFC were greater for API than ANI. There was a group by valence interaction effect in the right anterior insula in which PTSD subjects exhibited greater differential activation for ANI-API than did healthy controls. Regression analysis revealed that, within the PTSD group, more severe re-experiencing symptoms was related to *greater* differential left anterior insula activation, while severity of avoidance symptoms was related to *less* differential right anterior insula activation. More severe hyperarousal symptoms were related to less differential left amygdala and subgenual anterior cingulate (ACC) and greater differential superior and medial PFC activation.

Discussion: Results are consistent with findings from previous studies on anticipatory processing in that IPV-related PTSD was associated with greater differential anterior insula activation during anticipation of negative vs. positive stimuli as compared to healthy controls. Current findings suggest anterior insula activation during anticipation of negative vs. positive stimuli may depend on whether an individual's PTSD symptoms relate more to re-experiencing vs. avoidance. These findings are similar to findings from previous research related to re-experiencing vs. dissociative responses in PTSD. In conclusion, these results support the hypothesis that neural substrates of PTSD differ for varying subtypes of the disorder and that paradigms involving affective anticipation are useful in delineating such differences. *This project was funded by the following: VA Merit Award and NIMH-MH64122 (PI: Murray Stein, MD, MPH).*

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131. A multimodal study of working memory-related brain activation in premenstrual dysphoria

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Background: An emerging body of data has demonstrated that the gonadal steroid hormones estradiol and progesterone affect the function of neural systems relevant to working memory, particularly in the dorsolateral prefrontal cortex (DLPFC)¹. These hormones are also implicated in the etiology of premenstrual dysphoria (PMD), a disorder characterized by extreme mood changes concurrent with fluctuations in hormone levels during the menstrual cycle². Clinical studies suggest that PMD is likely an abnormal response to relatively normal hormone levels², but this has not been explored at the level of the brain across different imaging modalities. To investigate the pathophysiology of PMD with functional neuroimaging, we used H₂¹⁵O PET and 3T fMRI to measure working memory-related blood flow in conjunction with a hormone manipulation paradigm that pharmacologically induces temporary hypogonadism in young women and separately replaces estradiol and progesterone.

Methods: As part of a larger study of the effects of gonadal steroids on brain and behavior, PET and fMRI scans were obtained from healthy women and women diagnosed with prospectively confirmed PMD during each of three different hormone conditions: (i) ovarian suppression (i.e. hypogonadism) induced by the gonadotropin-releasing hormone agonist leuprolide acetate (Lupron), (ii) Lupron plus estradiol replacement, and (iii) Lupron plus progesterone replacement. Fourteen volunteers (mean age 39 + / - 6 years) and 14 patients (mean age 37 + / - 8 years) underwent PET scanning. During

each hormone condition, PET rCBF measurements (10mCi $H_2^{15}O$ /scan) were obtained during a series of fourteen 60-second scans, which alternated between a 0-back (obk) sensorimotor control task and a 2-back (2bk) working-memory task. In the fMRI study, 13 volunteers (mean age 37 \pm 7 years) and 13 patients (mean age 40 \pm 7 years) underwent two runs of the Nback obk/2bk task per hormone condition. Each scan consisted of seven 24-second blocks, alternating between obk and 2bk. A global assessment of function (GAF) score was obtained for each patient prior to entry into the pharmacological protocol. For PET, scans were anatomically normalized to an average template, smoothed (10 mm³ Gaussian kernel), and scaled to remove global blood flow variations with SPM5. For fMRI, data were preprocessed in SPM5 (registration, time slice correction, warping, smoothing at 10 mm). Though performed separately, group level analyses for both imaging modalities were identical. Using a random-effects analysis with subject as a dependent measure, between-group differences in working memory activation (2bk-obk) were assessed with an exploratory statistical threshold of $p < 0.001$, uncorrected. Correlations between average 2bk-obk activation and GAF scores were performed at a threshold of $p < 0.001$, uncorrected.

Results: For both fMRI and PET cohorts, there were no between-group differences in serum hormone levels. In PET, a main effect of group on working memory (WM) activation across all hormone conditions was observed, with patients showing greater DLPFC recruitment than healthy controls, specifically on the right. Patients' GAF scores positively correlated with activity in the precentral gyri and right middle temporal gyrus ($p = 0.001$). A negative correlation (greater cortical activation, worse [lower] GAF score) was seen bilaterally in the prefrontal cortex, including the middle frontal, medial frontal, and superior frontal gyri ($p = 0.001$). Analysis of fMRI data showed similar, robust findings, with a main effect of group on WM activation in the medial frontal gyrus ($p < 0.005$ FDR-corrected) and bilateral DLPFC significant at $p < 0.001$. In fMRI, patient's GAF scores positively correlated with bilateral middle temporal gyrus at $p < 0.001$ and negatively correlated with the medial frontal and middle frontal gyri at $p = 0.001$.

Discussion: Our results indicate that patients with PMD have abnormal patterns of blood flow during working memory, specifically in the DLPFC, and, moreover, that these neurophysiological abnormalities relate to symptomatic burden, as measured by the GAF, in these populations. The concordance of the fMRI and PET findings strongly suggest the validity of these findings. These data present a novel framework within which to explore the neural mechanisms underlying PMD, and further suggest that these mechanisms are functionally relevant within the context of working memory and its neural substrate.

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132. Resting regional cerebral blood flow patterns differentially predict neuropsychological performance in separable cognitive domains

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Background: The search for a neural substrate of higher cognitive functions occupies a substantial position in neuroimaging research. Such studies most commonly explore the state of the brain while participants are engaged in a task during scanning. In the present work, we sought to link different cognitive abilities to task-independent neurofunctional patterns by using the oxygen-15 water PET method for measuring regional cerebral blood flow (rCBF), a gold

standard approach to determining the resting neurofunctional landscape, and correlating rCBF values with factor-based composite scores for five cognitive domains (nback, card sorting (CS), verbal memory, processing speed, and span). These factor scores were derived from a comprehensive neuropsychological battery and have been previously shown to reflect separable cognitive realms².

Methods: Participants included forty right-handed healthy individuals, aged 20-50 (20 males, 20 females) who were found by way of history, physical exam, and the Structured Clinical Interview for DSM-IV to be free of medical and psychiatric illness, substance abuse, and pharmacological treatment. These individuals completed a battery of neuropsychological tests, and the five cognitive factors were calculated from these data². Separately from the collection of the neuropsychological data, rCBF data were acquired from the same cohort of forty subjects on a GE advance PET camera in 3D mode via two 60-second scans, each following an IV bolus of 10 mCi of oxygen-15 water, while participants rested with their eyes closed. PET data were analyzed using SPM5. Following registration, attenuation, and subtraction of background activity, images were anatomically normalized to an average PET template, scaled proportionally to a whole brain mean of 50 to remove variations in global blood flow, and smoothed using a 10 mm Gaussian smoothing kernel. After preprocessing, the two resting scans of each subject were averaged for group-level analysis.

Using the averaged PET data, we performed regression analyses with each of the five cognitive factors listed above ($p < 0.001$, uncorrected).

Results: Distinct patterns of correlation with resting rCBF emerged for each of the cognitive factors. Of particular interest, the nback and CS factors were associated with non-overlapping neural networks: nback correlated positively with rCBF in the left middle frontal gyrus ($R^2 = 0.37$), left inferior parietal lobule ($R^2 = 0.36$), left posterolateral temporal cortex ($R^2 = 0.35$), and left precuneus ($R^2 = 0.35$); whereas CS factor scores predominately showed negative correlation with resting rCBF in the right and left hippocampi ($R^2 = 0.36$ and 0.30 , respectively), as well as a small area of positive relationship within the right superior temporal gyrus ($R^2 = 0.36$).

Discussion: We identified regionally distinct relationships between rCBF and the five cognitive factors. That these correlations occurred between cognitive performance obtained independently of the scanning procedure and rCBF measured independently of cognitive tasks suggests that these patterns reflect trait-like differences in circuit-level, basal activity. The regional pattern of resting rCBF associated with higher nback factor scores specifically in dorsolateral prefrontal cortex and inferior parietal lobule reflects the pattern of activation observed during this task³. The positive correlation that we found between resting rCBF in these regions and nback factor scores may indicate that individuals with higher resting neural activity in these regions may tend to engage working memory more frequently at rest, and therefore may recruit this neural circuitry more effectively when called upon to do so during testing, leading to higher scores. In contrast, hippocampal activation is not typically observed during CS⁴, and in fact relative suppression of this region has been described¹. Thus, one interpretation of the negative association of resting hippocampal rCBF with CS factor scores is that individuals with higher resting hippocampal activity may tend to engage episodic memory systems more often during rest; they may also tend to rely on this hippocampally-dependent system during CS, rather than utilizing more task-appropriate executive and working memory systems, resulting in worse performance. However, further work is required to corroborate these results and to further explore the implications of our findings.

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133. Olfactocentric Paralimbic Cortex Volume in Adolescents with Bipolar Disorder

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Background: Olfactocentric paralimbic cortices are central in emotional regulation processes disrupted in bipolar disorder. These cortices undergo maturation during adolescence, implicating disturbances in the development of olfactocentric paralimbic cortices in bipolar disorder in adolescence. This study investigates cortical morphology in adolescents with bipolar disorder; reductions in olfactocentric paralimbic cortical volume were hypothesized.

Methods: Voxel-based morphometric analyses were performed to compare cortical gray matter on high-resolution structural magnetic resonance imaging scans of forty-one adolescents with bipolar disorder to those of seventy-seven healthy comparison adolescents.

Results: Volume was decreased in olfactocentric paralimbic cortex including orbitofrontal, insular and temporopolar cortices in adolescents with bipolar disorder, compared to healthy adolescents ($p < 0.001$). Volume was also decreased in adolescents with bipolar disorder, compared to healthy adolescents, in inferior prefrontal cortex, temporal association cortex and cerebellum.

Discussion: This study supports olfactocentric paralimbic cortex volume reductions in adolescents with bipolar disorder. There is a paucity of study on the development of these cortices in health and in psychiatric disorders. The findings suggests that further study of olfactocentric paralimbic cortical development may provide insights into the neurodevelopment of bipolar disorder.

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134. Altered Cerebral Gamma-Aminobutyric Acid Type A Benzodiazepine Receptor Binding in PTSD Determined by [¹¹C] Flumazenil Positron Emission Tomography

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Background: Patients with PTSD exhibit excessive physiological responses to everyday internal or external stimuli. This may reflect increased “bottom-up” activation of the amygdala or insufficient “top down” regulation, putatively associated with insufficient gamma-aminobutyric acid type A-benzodiazepine (GABAA-BZD) receptor activity. GABA is the main inhibitory neurotransmitter in the brain inhibiting glutamatergic, dopaminergic, serotonergic and noradrenergic pathways. The GABAA-BZD receptor is ubiquitously distributed throughout the brain, concentrated in gray matter. It has binding sites for GABA, barbiturates, benzodiazepines (BZD), anticonvulsants, and neuroactive steroids. Hypotheses regarding the role of GABAA/BZD receptor function in PTSD have proposed either changes in the GABAA/BZD macromolecular complex or alterations in its concentration. However, findings from current studies are conflicting.

Methods: Twelve medication-free outpatients with civilian PTSD (34.8 ± 10.2 years old, 8 women) and fifteen non-traumatized, healthy subjects (33.8 ± 10.5 years old, 9 women) participated in the study. Traumas occurred either prepubertally ($n=7$) or as adults ($n=8$). Psychiatric diagnoses were established using the Structured Clinical Interview for DSM-IV (SCID). Severity of PTSD was moderate, with a Clinician-Administered PTSD Scale (CAPS) score of 64.2 ± 13.7 . Depression (Inventory of Depressive Symptomatology (IDS) 20.8 ± 8.2) and anxiety (Hamilton Anxiety Rating Scale (HAMA) 13.4 ± 8.3) levels were moderate as well. PET scans were acquired using a GE Advance scanner with septa retracted (35 contiguous slices; 4.25 mm plane separation; reconstructed 3D spatial resolution =

6-7 mm full-width at half-maximum). A transmission scan corrected for attenuation. 20 mCi of high specific activity [¹¹C]-flumazenil was injected, with an upper limit to of 9 µg per 70 Kg. A 60-min dynamic emission image of the brain was initiated at injection. Structural MRI scans were acquired using a 3.0 Tesla scanner. PET images were registered to the individual's MRI with a mutual information algorithm. Binding potential (BP) images were created using the 2-step version of the simplified reference tissue model (SRTM2). Input kinetics for the reference tissue were derived from the pons, where [¹¹C]flumazenil binding predominantly reflects free and nonspecifically bound radiotracer. PET images were filtered using a 10-mm gaussian smoothing kernel. Using SPM2, the [¹¹C] flumazenil BP values were compared between groups in a voxel-wise analysis using a two-sample t-test model. Regional between-group differences in the mean BP were considered significant if the peak voxel t-value corresponded to $p_{\text{uncorrected}} \leq 0.005$ and the cluster-level p-value remained significant after applying corrections for multiple testing. Regions in which the cluster level p value did not remain significant after applying corrections for multiple testing were presented if they replicated results of previous benzodiazepine receptor imaging studies in PTSD. A correlation analysis assessed the association between the CAPS score and regional [¹¹C] flumazenil BP. Due to the exploratory nature of this analysis we applied a significance threshold of $p_{\text{uncorrected}} \leq 0.005$ without correction for multiple testing.

Results: Mean [¹¹C] flumazenil BP was significantly higher in patients with PTSD in the dorsomedial prefrontal cortex and precuneus. [¹¹C] flumazenil BP also was higher in PTSD in the left orbitofrontal cortex, though not surviving cluster correction. No areas were identified where the mean BP was reduced significantly in PTSD. CAPS scores correlated significantly with flumazenil receptor BP values in the left posterior and anterior insular cortices.

Discussion: Regions of the prefrontal cortex (PFC), including the orbitofrontal cortex mediate emotional regulation and reward processing. Increase in BZD BP could indicate functional impairment in these regions resulting in a compromised coping with emotionally demanding conditions and reward seeking. The precuneus has a central role in visuo-spatial imagery, episodic memory retrieval and self-consciousness. Increased BZD binding potential in the precuneus may relate to previous observations of hypoactivity in PTSD, in glucose metabolism and connectivity to the medial prefrontal cortex, thalamus, parietal and temporal cortices in chronic patients, and to the posterior cingulate cortex and amygdala after acute trauma (predicting the onset of PTSD). Increased BZD BP may contribute to hypoactivity in the precuneus and thus be compatible with the above findings.

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135. Neural Correlates Of Extinction Recall: Effects Of Development

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Background: Neuroimaging research in anxiety disorders has demonstrated perturbed function in amygdala-prefrontal cortex circuitry when confronting threats that signal impending danger. Functional MRI studies of extinction recall report ventromedial prefrontal cortex (vmPFC) and hippocampus activations (Milad et al., 2007; Milad et al., 2009). Fear and safety learning may interact with development in several important ways, which may predict the outcome of pediatric anxiety. Although the ability of the amygdala to generate conditioned fear responses likely emerges early (Bachevalier et al, 2001; Hunt et al., 1999), cortical regions reach maturity later in development (Gogtay et al., 2010). The capacity to discriminate “threat” from “safety” also may mature with development, such that failures to increase this discrimination capacity in childhood may contribute to persistent

anxiety disorders. Using a fear conditioning paradigm involving a “screaming lady,” we examined extinction recall in healthy adolescents and healthy adults to test the hypothesis that group differences occur in subjective response and vmPFC activation in response to “threat” vs. “safety” cues.

Methods: To date, 24 healthy adolescents (13.8 ± 2.7 years, 11 males) and 26 healthy adults (27.3 ± 6.9 years, 11 males) participated in a fear conditioning experiment, involving fear acquisition and extinction, and returned to complete extinction recall in a 3T MRI scanner (Britton et al., 2010). Two pictures of female faces displaying neutral expressions served as conditioned stimuli (CS+ and CS-) and a “screaming lady” served as the unconditioned stimulus (US). Throughout the experiment, psychophysiological monitoring was recorded. Following fear acquisition and extinction, subjects reported their anxiety to each CS. During extinction recall, subjects viewed morphed images continuously varying from the CS- to CS+. Participants made yes/no judgments about each morphed image by button press. Three questions probed explicit memory, emotional state, and perceptual discrimination. Preprocessing and whole-brain, voxel-wise, random-effects analyses were conducted in AFNI. Significant condition effects (CS+ vs. CS-) were identified and t-tests will examine between group differences using a $p < 0.005$ uncorrected threshold.

Results: Following fear acquisition, a conditioning effect based on subjective ratings was detected [$p < 0.001$]. Adolescents reported more anxiety overall [$p < 0.05$]; however, there were no group differences in the extent of conditioning [$p > 0.8$]. Following extinction, anxiety ratings to the CS+ decreased in the healthy adults [$p < 0.009$] but not the healthy adolescents [$p > 0.3$]. However, at the end of extinction, the EMG startle response indicated that there was no difference in response between CS+, CS-, and ITI [$p > 0.2$], and no group effects [$p > 0.1$]. A previous analysis in 13 healthy adolescents showed that during extinction recall, adolescents were successful in discriminating the CS+ and CS- based on explicit memory and emotional state and exhibited vmPFC activation [Talairach x, y, z: -5, 29, -14] to the CS+ vs. the CS- when assessing threat appraisal. The percent signal change values extracted from this region indicated a quadratic response [$R^2 = 0.9$], indicative of a generalization gradient. Data collection is ongoing. Group differences in subjective and vmPFC activation during extinction recall will be investigated using similar methods.

Discussion: During extinction recall, vmPFC activation, showing a generalization gradient between “safety” and “threat”, was detected when adolescents were appraising threat, consistent with the few neuroimaging studies in healthy adults. Developmental differences in activation of the vmPFC during threat appraisal during extinction recall may reflect the normal maturation of threat-safety classification. Deviations from the normal maturation trajectory of vmPFC function and threat-safety classification ability may allow the identification of sensitive periods for clinical expression of anxiety disorders.

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136. Differences in Striatal D2/D3 Dopamine Receptor Availability Associated with Cigarette Smoking

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Background: The role of the mesolimbic dopaminergic system in the actions of nicotine and other drugs of abuse is well established, with evidence for lower dopamine (DA) receptor availability associated with various addictions. In a previous study (Fehr et al, 2008), nicotine-dependent male smokers (>15 cigs per day), independent of withdrawal status, showed significantly lower availability of dopamine (DA) D2/D3 receptors in the putamen when compared to male nonsmokers. Female subjects were not studied. The goal of our study was

to extend this work by comparing male and female smokers and nonsmokers.

Methods: We measured DA D2/D3 receptor availability in 19 smokers (10 men, mean cigarettes/day = 13.55 ± 6.84 ; 9 women, mean cigarettes/day = 13.17 ± 7.44) about 1 h after each smoked a cigarette, and in 17 age-matched nonsmokers (8 men; 9 women), using positron emission tomography (PET) and the D2/D3 receptor radioligand [18 F]fallypride. Participants also received a 1.5T T1-weighted MRI scan. The Simplified Reference Tissue Model 2 in PMOD 3.1 was used to calculate putamen binding potentials (BP). Statistical analyses included 2-way ANOVA to assess the effects of sex, smoking status and the interaction between them on putamen BP. The primary hypothesis, that smokers have lower putamen BP than nonsmokers irrespective of sex, was tested using one-tailed unpaired t-tests.

Results: There were no significant differences between the groups in age and, for smokers, cigarette consumption. Male smokers were low to moderately nicotine-dependent (mean FTND 3.2 ± 2.0) as were female smokers (mean FTND 3.3 ± 1.5). Mean BP for male smokers (nonsmokers) was 17.17 ± 2.76 (19.06 ± 2.84) and for female smokers (nonsmokers) was 22.2 ± 4.71 (19.8 ± 4.65). The 2-way ANOVA showed no significant main effects or interactions. For males the group difference between smokers and nonsmokers was 1.43 (one-tail, $p = 0.08$), compatible with the previous result but not independently significant for lower putamen BP. For females t was -1.09 (one-tail $p = 0.15$) although the mean was higher for the smokers.

Discussion: The observation of lower DA D2/D3 receptor availability in the putamen of male smokers (non-significant) is compatible with the previous finding. Lack of significance in the sample studied to date may reflect less smoking exposure. Additional work is needed for rigorous assessment of our preliminary finding and to test whether there are sex-related differences in the interaction of smoking with striatal D2/D3 DA receptors. Previous studies showing that women have significantly greater cortical DA release while men have greater striatal DA release following an amphetamine challenge (Riccardi et al, 2006), and that women have higher striatal DA transporter levels than men (Staley et al, 2001) suggest that sex-related differences may affect the CNS response to nicotine.

References: Fehr C et al. (2008). Association of low striatal dopamine D2 receptor availability with nicotine dependence similar to that seen with other drugs of abuse. *Am J Psychiatry* 165:4

Riccardi P et al. (2006). Sex differences in amphetamine-induced displacement of [18 F]fallypride in striatal and extrastriatal regions: a PET study. *Am J Psychiatry* 165:9

Staley J et al. (2001). Sex differences in [123 I]beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and non-smokers. *Synapse* 275-284

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137. Increased frontal functional connectivity in cocaine-dependent subjects and its association with delayed discounting and reversal learning task performance

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Background: Functional neuroimaging studies suggest that chronic cocaine use is associated with frontal lobe deficits in regional cerebral blood flow and brain glucose metabolism levels. In addition, diffusion tensor imaging has provided evidence that cocaine abuse is associated with altered frontal white matter connectivity. Functional connectivity in gray matter of cocaine-dependent subjects, however, has not been examined yet. This is the first study to our knowledge that examines functional connectivity of anterior cingulate cortex (ACC) during rest in cocaine-dependent subjects. Because ACC is known to integrate inputs from different brain regions to regulate behavior, we hypothesize that cocaine-dependent subjects will have connectivity

abnormalities in ACC networks. In addition, we hypothesized that connectivity abnormalities would be associated with poor performance in delayed discounting and reversal learning tasks.

Methods: Resting functional magnetic resonance imaging data was collected to look for functional connectivity differences between twenty-seven chronic cocaine-dependent subjects (5 females, age: $M=39.73$, $SD=6.14$) and 24 controls (5 females, age: $M=39.76$, $SD=7.09$) were recruited. All participants were also assessed with delayed discounting and reversal learning tasks. Using seed-based functional connectivity measures, we examined functional connectivity in cocaine-dependent subjects and controls within five ACC connectivity networks with seeds in subgenual, caudal, dorsal, rostral, and perigenual ACC.

Results: Cocaine-dependent subjects showed increased functional connectivity within the perigenual ACC network in left middle frontal gyrus, ACC and middle temporal gyrus when compared to controls. Functional connectivity abnormalities were significantly positively correlated with task performance in delayed discounting and reversal learning tasks in cocaine-dependent subjects. CU with increased FC between perigenual ACC and DLPFC showed both increased compromise when learning to reverse reward contingencies (manifested with higher number of trials before a reversal) and increased levels of impulsivity (manifested with higher delay k values).

Discussion: The present study shows that participants with chronic cocaine-dependency show hyperconnectivity within an ACC network known to be involved in social processing and mentalizing. In addition, these abnormalities were associated with difficulties to delay rewards and slower adaptive learning found in cocaine-dependent subjects.

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138. Neuromagnetic Response of Fusiform Gyrus in Early Emotional Face Processing

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Background: In recent years, many brain regions comprising a complex neural network involved in emotional face processing have been identified. Although the fusiform gyrus (FFG) in the ventral temporal lobe plays an important role in face processing, it remains unclear whether early fusiform activity differs when processing faces with different emotional expressions. Taking advantage of the high temporal and spatial resolution of magnetoencephalography (MEG), this study examined early FFG face processing elicited by different emotional facial expressions.

Methods: Seventeen subjects without history of psychiatric or neurological disorder participated. Emotional facial expressions (angry, disgust, fear, happy, sad) and neutral faces were randomly presented for 1.3 sec with a 1.5 sec inter-stimulus interval. Stimuli were derived from the Ekman Pictures of Facial Affect, and each emotional expression consisted of 144 images. MEG data was obtained using a 122-channel whole-cortex MEG. Vector-based Spatial-temporal Analysis using an L1-minimum-norm (VESTAL) provided measures of early FFG activity (< 200 ms), with results visualized using FreeSurfer software. Emotion \times Hemisphere ANOVA examined the latency and source strength of peak FFG activation. t -contrast maps were also created to compare responses in the FFG between emotional expressions and neutral faces.

Results: Across all emotions, right FFG showed significantly greater activation as well as shorter latencies than left FFG ($p_{\text{fear}} > \text{happy} > \text{sad} > \text{anger} > \text{neutral}$), with t -contrast maps ($p < 0.001$) revealing stronger right FFG activation to all emotional facial expressions than neutral faces, excluding the sad condition.

Discussion: Our findings of stronger right than left FFG activation suggest that right hemisphere is more dominant in the early perception

of emotional expression. The lack of significant differences in FFG activation between sad and neutral faces may be due to the less arousing effect of sad expressions. VESTAL source localization data suggests there is some specificity to facial expressions in areas of the FFG.

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139. Neural Correlates of Rapid Antidepressant Response to Ketamine in Bipolar Depression

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Background: Multiple lines of evidence implicate a role for the glutamatergic system in the pathophysiology and treatment of mood disorders. Consistent with this hypothesis, the N-methyl D-aspartate (NMDA) antagonist ketamine has been shown to induce a rapid and significant reduction in depressive symptoms in subjects with bipolar depression. The neural mechanisms behind this effect are unknown. The present study utilizes [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) to examine the metabolic response to ketamine infusion in subjects with bipolar depression. Because the [^{18}F]-FDG PET measure of glucose metabolism is dominated by the uptake of glucose into glia in response to neuronal glutamate release, we expect the metabolism to reflect the influence of ketamine on glutamate transmission.

Methods: Twelve currently depressed subjects with bipolar disorder [on stable monotherapy with either lithium ($n=9$) or divalproex ($n=3$)] participated in PET imaging during a double-blind, randomized, crossover treatment study in which they received ketamine (0.5 mg/kg IV over 40 minutes) and placebo (saline) infusions on separate occasions, separated by 2 weeks. Subjects received 4.5 mCi of [^{18}F]-FDG 120 minutes after the end of each infusion, and PET scans were obtained on a GE Advance scanner. Structural MRI scans were performed to provide an anatomical framework for PET data analysis. Regional metabolic rate for glucose (rMRGlu) was calculated using an image-derived cardiac input function and venous blood glucose samples. Mean rMRGlu measurements were derived from pre-determined regions of interest placed on individual subject MRI and masked with a map of gray matter. Mean values were normalized by whole brain metabolism. The Montgomery-Asberg depression rating scale (MADRS) was administered at baseline and 230 minutes post-ketamine infusion, and the percent change in score was correlated with the change in rMRGlu. A voxel-wise whole brain analysis of raw radioactive count images ($N=12$) was performed in SPM5.

Results: Whole brain metabolism did not differ between the placebo and ketamine conditions ($p=0.52$). Compared to baseline, rMRGlu following ketamine infusion was increased in the ventral striatum ($p=0.03$). The change in rMRGlu negatively correlated with the percent change in MADRS scores in the right (R) ventral striatum ($r=-0.64$, $p=0.02$), and positively correlated with the percent change in MADRS scores in the left (L) ventrolateral prefrontal cortex (PFC) ($R=0.65$, $p=0.02$). In the voxel-wise analysis, metabolism increased post-ketamine in the L inf. and sup./middle temporal gyri (STG/MTG), L motor cortex (C), mid- and posterior cingulate, R superior temporal G, L frontopolar C (FPC), R amygdala, and dorsal ACC ($p \leq .001$, uncorrected). The findings in L STG/MTG remained significant after correction for multiple comparisons. Subjects had metabolic decreases post-ketamine (relative to placebo) in L lat. cerebellum, medial cerebellum, L and R dorsolateral PFC, R Occip C, R inf. parietal C, R retrosplenial C, R FPC, L ventral claustrum/striatum, R STG/MTG, R post. putamen/lat. thalamus, R insula, L middle temporal G, L lat. orbitofrontal C (OFC), R motor C, R sup. frontal G, L sup. parietal C, and R post. OFC ($p \leq .001$, uncorrected). The L lat. and medial

cerebellar findings remained significant after correcting for multiple comparisons.

Discussion: Metabolism increased under ketamine in the ventral striatum, and this increase correlated with improvement in depressive symptoms. Conversely, decreased metabolism in ventrolateral PFC correlated with clinical improvement. These and other structures we found with altered metabolism under ketamine form an extended “visceromotor network” that modulates autonomic, endocrine, behavioral and experiential aspects of emotional behavior. Increased activity in the ventral striatum has previously been reported in bipolar depression. Functional imaging studies of standard antidepressant treatments have demonstrated treatment-induced *decrease* of activity in ventral striatum, amygdala, and subgenual cingulate. The ketamine-induced *increases* we observed in these areas suggest that ketamine has unique effects on neurocircuitry compared with traditional antidepressant treatments, possibly by increasing glutamatergic transmission through non-NMDA receptors. Further study of the neural mechanisms by which ketamine brings about rapid antidepressant effects is likely to provide key information about the pathophysiology of bipolar depression and guide development of novel treatments.

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140. Default Network Structure and Resting-State Functional Connectivity: Searching for the Neural Correlates of Obsessions and Compulsions in Autism Spectrum Disorders

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Background: Autism Spectrum Disorders (ASD) are characterized by disturbances in social function and communication, and the presence of repetitive behaviors. In addition, ASD is sometimes associated with other co-morbid symptoms, including obsessions and compulsions. These symptoms are reported in 37-95% of individuals with autism and have been observed to share some overlap with characteristic symptoms of obsessive-compulsive disorder (OCD). Given these observations, both ASD and OCD are hypothesized to share similar neurocognitive circuits that may underlie the presence of obsessions and compulsions in both disorders. The literature has suggested a possible role for default network dysfunction in ASD and OCD. We hypothesized that an alteration of the functional interplay between structures of the default network may underlie obsessive and compulsive symptoms observed in children with ASD. Furthermore, we hypothesized that atypical resting-state functional connectivity will reflect differences in structural connectivity between ASD individuals exhibiting high vs. low obsessive-compulsive symptoms.

Methods: 35 ASD children (ages 10-18) and 35 controls matched for age, gender and IQ took part in this functional MRI (fMRI) and diffusion tensor imaging (DTI) study. The Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Schedule were used to assist in the ASD diagnosis. Obsessive and compulsive symptoms were evaluated by using the Obsessive-Compulsive Scale (OC scale) of the Child Behavior Checklist. Categorization into ASD (high obsessive-compulsive symptoms) or ASD (low obsessive compulsive symptoms) groups was based on OC scale scores: patients with scores <8 were categorized as having “low symptoms,” whereas patients with scores >8 were categorized as having “high” symptoms. During fMRI acquisition, participants were instructed to “let your mind wander freely” while looking at a fixation cross displayed in the middle of the

screen for 10 minutes during fMRI acquisition. A seed region was placed in the PCC and functional connectivity was examined by obtaining the correlational activity between the posterior cingulate cortex (PCC) and other areas of the default network. Meanwhile, a voxelwise analysis of multi-subject diffusion data was pursued using TBSS (Tract-Based Spatial Statistics).

Results: Initial efforts in our lab (including a sample of 12 adolescents with ASD and 12 controls) indicated that there are differences in default network functional connectivity between adolescent ASD individuals with high vs low obsessive-compulsive symptoms. Increased obsessive and compulsive symptom severity, as evidenced by higher OC Scale score, was found to correlate with decreased functional connectivity between the bilateral parahippocampal gyrus, the bilateral angular gyrus, the superior frontal gyrus, and the PCC. Diffusion tensor imaging results are to follow.

Discussion: Preliminary results show group differences in default network functional connectivity between adolescent ASD individuals with high vs low obsessive-compulsive symptoms. Upcoming analyses will yield additional information on the functional and structural connections between default network structures thought to underlie obsessive and compulsive symptoms associated with ASD. Significance: The study of comorbidity in ASD is of utmost importance, given that it will provide new knowledge on behavioral differences that currently complicate research and treatment of this disorder. Furthermore, by deconstructing ASD into sets of simpler traits that are intermediate along the pathway that links vulnerability genes and disease, we aim to better understand this complex and heterogeneous disorder. At present, relatively little is known about the processes that contribute to the presence of individual differences in co-occurrence of symptoms in ASD. This study aims to address this gap in the literature.

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141. Attenuated Amphetamine-Induced Dopamine Release in Subjects at High Familial Risk for Substance Dependence

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Background: Drugs of abuse across pharmacological classes increase meso-striatal dopamine transmission. Indirect evidence from animal models and studies in people with current addiction disorders suggest that this response might be disturbed in those at risk for addiction. To investigate this possibility, we measured striatal dopamine responses to a drug challenge in young adults with a multigenerational family history of substance use disorders.

Methods: Three groups were tested. 1) Family history positive (FH-Pos): 16 non-dependent subjects (age: 21.3 ± 2.4 y.o.) with a multi-generational FH of substance abuse (1.6 ± 0.6 1st degree relatives, 2.9 ± 1.1 2nd degree relatives) and a personal history of cocaine or amphetamine use (49.6 ± 59.3 stimulant uses). 2) FH-Neg: 15 subjects (age: 21.7 ± 1.9 y.o.) with no 1st or 2nd degree relatives with substance use problems, matched to FH-Pos on their personal history of drug use (43.5 ± 40.7 stimulant uses). 3) Control (CTRL): 19 healthy controls without substance abusing relatives or a personal history of stimulant drug use (age: 20.5 ± 2.2 y.o.). All subjects had two PET [11 C]raclopride scans. Sixty minutes before each scan they were administered 0.3 mg/kg d-amphetamine p.o. or placebo, given double-blind. Visual analogue scales were used to monitor subjective responses.

Results: FH-Pos subjects had a smaller [11 C]raclopride response to amphetamine than either FH-Neg or CTRL groups, and these two control groups did not differ from one another. The differential

response was most pronounced in the right ventral striatum where amphetamine decreased [¹¹C]raclopride binding values by $15.7 \pm 15.0\%$ in CTRL, $14.3 \pm 8.4\%$ in FH-Neg, and only $3.4 \pm 12.6\%$ in FH-Pos ($p = 0.006$ vs. CTRL, $p = 0.021$ vs. FH-Neg). Subjective responses to the amphetamine challenge did not differ between the three groups with the exception of 'Want Drug' which was significantly greater in the subjects at risk for addiction ($p = 0.031$ vs. CTRL, $p = 0.007$ vs. FH-Neg).

Discussion: The present study suggests that subjects at familial risk for addiction have diminished dopaminergic responses to d-amphetamine. The effect remained after controlling for past drug use, was accompanied by elevated drug-induced drug craving, and resembled that reported in currently dependent alcoholics and cocaine addicts. Since the present study's subjects were non-dependent, low dopamine responsiveness appears to express itself well before extensive drug exposure, potentially reflecting a trait marker for addiction.

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142. Genetic Contributions to Brain Intrinsic Functional Architecture: A Twin Study

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Background: Twins studies show genetic factors strongly influence brain volumetric measures (Schmitt et al., 2009; Thompson et al., 2001). By contrast, the genetic architecture of brain function is only beginning to be examined. A sample of 60 twins pairs performing the N-back working memory task yielded correlations between monozygotic (MZ) twins that were approximately twice those in the dizygotic (DZ) pairs resulting in non-significant heritability estimates of 14-30% (Blokland et al., 2008). Analyses of low-frequency spontaneous BOLD fluctuations (resting-state fMRI = R-fMRI) provide intricate maps of functional circuits that are stable over intervals as long as 5-16 months (Shehzad et al., 2009). A recent analysis of an extended pedigree found default network resting state functional connectivity heritability = 0.42 ($p = .005$; Glahn et al., 2010). Here we present initial results of R-fMRI analyses for 79 MZ twin pairs and 84 DZ pairs.

Methods: Image preprocessing (FSL and AFNI): 1) slice timing correction, 2) motion correction, 3) temporal despiking, 4) spatial smoothing (FWHM = 6 mm), 5) 4D global mean-based intensity normalization, 6) temporal filtering (0.01-0.1 Hz, for correlation and homotopic analyses only), 7) linear detrending, and 8) nonlinear transformation to MNI152 space. Voxel-wise nuisance signal correction included: global signal, WM, CSF, 6 motion parameters, age, sex, and extent of registration 'error.' Voxel-wise heritability analyses were limited to a gray matter mask defined by $p \geq 25\%$. Analyses were Bonferroni corrected for multiple ROIs and Gaussian random field theory corrected for whole brain comparisons ($Z > 2.3$, $p < 0.05$). Heritability Estimation: Falconer heritability (H^2) = $2 * (\text{corr}[MZt1, MZt2] - \text{corr}[DZt1, DZt2]) = 2 * (MZr - DZr)$ with upper bound (H^2) = $\text{corr}[MZt1, MZt2]$. R-fMRI Measures: Seed-Based Correlations: Seed Selection. (A) We created eleven 7.5 mm radius seeds centered at each of the 11 left hemisphere (or midline) coordinates identified by Andrews-Hanna et al., (2010) as key nodes of default network sub-networks (default network core; dMPFC subsystem; medial temporal lobe subsystem). (B) Nine seeds (odd numbered superior and inferior seeds) sampled the anterior cingulate cortex (ACC; Margulies et al., 2007). (C) Four seeds (4, 6, 14, and 17) sampled the four functional divisions of precuneus/posterior cingulate cortex (Margulies et al., 2009). (D) Six sets of seeds, 3 in each caudate and 3 in each putamen were examined (Di Martino et al., 2008).

Amplitude: A voxel-wise measure, fractional amplitude of low frequency fluctuation (fALFF = total power < 0.1 Hz/total power in the spectrum) (Zuo et al., 2010). Voxel-Mirrored Homotopic Correlation: We computed homotopic RSFC between each voxel in the right hemisphere and its homologous voxel in the left using Pearson's r (z-transformed).

Results: Significant heritability (range 0.52-0.66) was detected for resting state functional connectivity with nine of the 11 default network seeds, for all nine ACC seeds, for three of four precuneus seeds in the left and all four precuneus seeds in the right hemisphere, and for all six striatal seeds bilaterally. Voxel-based indices (fALFF and VMHC) also revealed significantly heritable clusters in orbitofrontal and parietal-occipital regions (fALFF) and in primary cortex (VMHC).

Discussion: We confirmed that genetic factors influence the patterns of spontaneous fluctuations in brain activity and that these can be detected within individual voxels/regions and in circuits defined on the basis of functional connectivity with high spatial precision. We speculate that the relationships observed represent the functional scaffold which forms the basis for neural development through interaction with the environment. The observed statistical maps provide specific hypotheses for planned GWAS analyses and for comparison with a human gene expression data set provided by the Allen Institute for Brain Science.

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143. Comparison of Amygdala Functional Connectivity During Mania Versus Depression in a Longitudinal Sample of Patients with Bipolar Disorder

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Background: Bipolar disorder (BPD) is a serious psychiatric illness that affects approximately 1.5% of the U.S. population and represents a significant source of individual morbidity and societal cost. Prior neuroimaging studies in BPD suggest there is potential breakdown in cortico-limbic regions responsible for emotional homeostasis. However, the majority of these studies are cross-sectional and there have been few prior longitudinal functional imaging studies examining patients across different mood states. Comparing the same patient group across manic and depressive episodes may provide specific neural correlates for each mood state and lead to a better understanding of the workings and breakdown of cortico-limbic circuits that generate mood.

Methods: Fifteen patients with BPD were recruited from the University of Cincinnati Academic Health Center during an acute manic episode. Following informed consent, all subjects were evaluated using the Structured Clinical Interview for DSM-IV. Subjects received an MRI scan during the index manic episode and then a second scan during a depressive episode (on average 14 weeks after the initial scan). All subjects were scanned at the University of Cincinnati College of Medicine's Center for Imaging Research (CIR) using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA). During both MRI scans subjects performed a Continuous Performance Task with Emotional and Neutral Distracters (CPT-END). The CPT-END task involves a visual oddball paradigm with the addition of emotional and neutral pictures taken from the International Affective Picture System (IAPS; University of Florida). A voxel-wise functional connectivity analysis was then performed using the left and right amygdala seed regions.

Results: The fifteen subjects had an average age of 30 and an average of 14 years of education. All subjects were medicated during the scans and were taking at least one mood stabilizer or an atypical antipsychotic (6 subjects were taking both classes of medication). There were no significant differences in accuracy (squares $F = 2.2$, $P = 0.15$, circles

$F=1.0$, $p=0.32$, neutral images $F=1.2$, $p=0.27$, emotional images $F=2.7$, $p=0.11$) or reaction time (squares $F=0.16$, $P=0.70$, circles $F=0.72$, $p=0.41$, neutral images $F=0.95$, $p=0.34$, emotional images $F=0.34$, $p=0.57$) in the CPT-END task between patients in manic and depressive periods. A functional connectivity analysis revealed increased correlation between the right amygdala and the right insula ($p<0.05$ corrected with a voxelwise threshold of 0.01 and a cluster threshold of 37 or more contiguous voxels) during depression.

Discussion: The current study is the first longitudinal comparison of functional brain activation across depressed and manic episodes that we are aware of. Patients showed increased functional connectivity between the right amygdala and right insula during depression. Prior neuroimaging studies have shown functional differences in the amygdala across mood states and structural and functional changes in the insula specific to bipolar depression. Given that the insula and amygdala have both been shown to be involved in emotional processing, increased connectivity between the two regions could represent dysregulation in the cortico-limbic system specifically linked to the generation of depressive symptoms.

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144. Effect of 5-HTTLPR Short Allele on Amygdalar and Hippocampal Volumes in Youth at High-Risk for Bipolar Disorder

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Background: Pediatric patients with bipolar disorder (BD) have been found to have decreased amygdalar and hippocampal volumes compared with healthy controls (Bearden et al., 2009; Pfiefer et al., 2008). To determine whether these abnormalities exist before onset of pediatric BD, we examined amygdalar and hippocampal volumes using magnetic resonance imaging (MRI) in youth at high risk for BD and healthy control subjects. We hypothesized that we would observe reduced amygdalar and hippocampal volumes in high-risk subjects. Furthermore, as the 5-HTTLPR s-allele has been associated with both BD and decreased amygdalar volume, we hypothesized that high-risk youth with an s-allele would have particularly reduced amygdalar and hippocampal volumes.

Methods: 37 children and adolescents at high risk for BD ("Prodromal Offspring", PO) and 23 age, handedness, and IQ matched healthy control (HC) subjects were included. Inclusion criteria for PO subjects were: age 9-18 years; at least one biological parent with BD I or II; and having a diagnosis of either MDD and/or ADHD plus moderate mood symptoms. All healthy control subjects had no current or past DSM-IV psychiatric diagnosis and did not have a first or second degree relative with any psychiatric diagnosis. All children were interviewed by masters-level researchers using the WASH-U-KSADS and parents were interviewed using the SCID. MRI images were collected on a 3 T GE Signa scanner and volumetric image analysis was performed using SPM8, BrainImageJava software and manual tracing of amygdalar and hippocampal regions. DNA was extracted from 200 µl of frozen blood and analyzed for the 5-HTTLPR short (s) - or long (l) - allele.

Results: 37 PO subjects (13.2 +/- 2.9 years, 26 males) and 23 HC subjects (13.2 +/- 2.9 years, 11 males) were included in the analysis. 25 subjects in the PO group had a diagnosis of major depressive disorder (MDD) and 21 had ADHD. After controlling for total brain volume and

age, there were no significant differences in amygdalar volumes in the PO compared with the HC groups. However, there was a trend for the PO group to have decreased total hippocampal volume ($p<0.07$). Presence of the 5-HTTLPR s-allele was found to contribute significantly to the variance in hippocampal volume ($p<0.01$) in an interaction with group status (PO vs. HC; $p<0.05$). No such effect of s-allele was found on amygdalar volume within each group and with all subjects overall.

Discussion: We did not find youth at high-risk for BD to have reduced amygdalar volume. However, reductions in hippocampal volume may be mediated by high-risk status as well as presence of the 5-HTTLPR s-allele. Similar to previous findings in youth at risk for depression (Chen et al., 2010), reduced hippocampal volume in this population may be a predisposing factor for BD. To clarify risk factors for BD development, PO subjects in this study will be followed longitudinally to determine progression towards BD.

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145. Test-retest reliability of paired-click 100 ms response in individuals with schizophrenia

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Background: Suppression of the 50 and 100 ms auditory evoked response to the second of two clicks in a paired-click task is considered a measure of sensory gating. Although impaired paired-click gating has been suggested as a schizophrenia endophenotype (i.e., increased P50 second click (S2)/first click (S1) ratio scores), most studies have unfortunately shown very low reliability for EEG P50 ratio scores. Other paired-click measures have been observed to be abnormal in individuals with schizophrenia, with most studies reporting a reduced 100 ms S1 response and increased ratio scores. A reduced 100 ms response is thought to reflect an encoding deficit in schizophrenia (e.g., Clementz et al., 2003; Edgar et al., 2008). Observing decreased N100 S1 amplitudes in a large sample of patients with schizophrenia as well as in a subset of their first-degree relatives, Turetsky et al. (2008) suggested N100 S1 amplitude as a candidate schizophrenia endophenotype. Smith et al. (2010) also observed decreased 100 ms first-click responses in patients with schizophrenia (EEG Cz and MEG left and right superior temporal gyrus [STG]) and showed that increased 100 ms ratio scores were clinically significant, with decreased 100 ms S1 responses associated with lower scores on tests of attention. Although the 100 ms S1 response appears promising as an endophenotype, to our knowledge the reliability of the EEG and MEG 100 ms response has not been established in individuals with schizophrenia. In the present study, EEG and MEG were simultaneously obtained across three sessions in individuals with schizophrenia and the reliability of EEG (Cz) and MEG (left and right STG) 100 ms paired-click responses was computed.

Methods: The standard paired-click task was administered to nine patients with schizophrenia in three sessions, with each session separated by at least one week. Cz EEG and whole-cortex MEG were simultaneously obtained. Cz and source-localized left and right STG measures were obtained for 100 ms S1 and S2 amplitude and latency, as well as for the S2/S1 ratio score. Reliability was assessed using intraclass correlation (ICC).

Results: For Cz N100, high ICCs were obtained for all amplitude measures (ICCs > 0.75), but not for S1 and S2 latencies (ICCs < 0.26). For left and right M100, high ICCs were obtained for all measures (ICCs for S1 > 0.77; ICCs for ratio scores > 0.79) except for left S2 amplitude (ICC = 0.58).

Discussion: In general, in patients with schizophrenia, good reliability was observed for the EEG and MEG 100 ms measures. Most importantly, ICCs greater than 0.75 were observed for EEG and MEG S1 amplitudes. ICCs measures at 0.7 above are considered sufficient for longitudinal studies of individual differences. As reliability is one of the criteria for a biological marker to qualify as an endophenotype, present findings provide additional support for 100 ms S1 paired-click amplitude as a schizophrenia endophenotype.

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146. Amphetamine-Induced Dopamine Release in Treatment-Naïve Adults with ADHD: a PET/[11C]Raclopride Study

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Background: Converging evidence suggests a dysfunction in dopamine (DA) neurotransmission in attention deficit/hyperactivity disorder (ADHD): the DA system plays an important role in a range of behaviors affected in ADHD, DA genes are implicated in ADHD's aetiology, and DA augmenting agents, such as methylphenidate (MPH) and dextroamphetamine (d-AMP) produce significant symptom improvement. Neuroreceptor imaging studies have provided the most direct evidence of DA dysregulation in ADHD, suggesting abnormalities in DA D2 receptor and DA transporter binding. Responsivity of the DA system to stimulant drug challenge has been investigated by two studies with somewhat conflicting results: one reported blunted MPH-induced DA responses in adults with ADHD (Volkow et al, 2007), whereas the other presented evidence that measures of inattention and impulsivity are associated with greater MPH-induced DA responses in adolescents (Rosa-Neto et al, 2005). The current study aimed to resolve the conflicting findings by investigating DA release in treatment-naïve adults with ADHD in response to a challenge with d-AMP.

Methods: Fifteen treatment-naïve men with ADHD (mean age 29.87) and fifteen healthy male controls (mean age 24.87) underwent two [11C]raclopride PET scans given double-blind: one following d-AMP (0.3 mg/kg, p.o.) and the other following placebo. [11C]Raclopride binding to dopamine D2/3 receptors following d-AMP and placebo administration was assessed in three regions of interest (ROIs) based on functional organization of the striatum (Martinez et al, 2003): limbic, associative, and sensorimotor subregions. A battery of neurocognitive tasks evaluating executive functions, impulsivity, and motor activity, as well as personality questionnaires were administered to all participants, and symptom severity was assessed with the Conners Adult ADHD Rating Scale (CAARS).

Results: As expected, compared to the healthy controls, the ADHD group had significantly higher symptom scores (CAARS ADHD index: $p < 0.001$) and performed more poorly on tasks of response inhibition, showing longer inhibitory reaction times on the stop signal reaction time task ($p = 0.008$), a higher error rate on the antisaccade task ($p = 0.006$), and a higher error rate on a version of the go/no-go task ($p = 0.05$). The ADHD group also showed greater d-AMP induced [11C]raclopride responses relative to controls across all three ROIs ($p = 0.03$), with the most pronounced difference in the sensorimotor subregion ($p = 0.006$). In addition, a quadratic inverted U relationship was observed between the d-AMP induced [11C]raclopride response in sensorimotor striatum and CAARS hyperactivity scores across both groups, with the largest [11C]raclopride response in individuals reporting moderate levels of activity and smaller responses in both non-hyperactive and highly hyperactive individuals ($r = 0.56$; $p = 0.009$).

Discussion: The findings are consistent with a model proposing abnormally low striatal DA tone coupled with an exaggerated phasic

DA release in ADHD (Grace, 2001), with greater increases in extracellular DA in the ADHD group likely reflecting the exaggerated phasic component. Stimulant medications might increase DA tone and diminish phasic reactivity. Since the most severely affected patients had lower DA responses, the inverted-U association is also consistent with reports that the clinical response to stimulants is greatest in patients with the most severe ADHD (Sahakian & Robbins 1977; Robbins & Sahakian 1979; Buitelaar et al 1995). Though limitations of the study include a relatively small sample size and inclusion of only male participants, the results support previous findings of altered DA response to stimulant challenge in ADHD and extend them by suggesting that this response may vary as a function of symptom severity.

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147. Optimizing fMRI feedback for training cognitive control: adding regional localization to a whole-brain classifier

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Background: For most of us, most of the time, the ongoing activity of the organ responsible for all that we think and feel is hidden to us - and not under voluntary control. We are controlled by our brain, not the other way around. However, brain feedback studies are changing this long-standing, one-way relationship: pioneering studies show that when individuals are given visual feedback about the activity of a brain region [based on blood oxygenation level-dependent (BOLD) signal, using functional magnetic resonance imaging, fMRI], they can often gain some voluntary control over the activity - offering the potential for brain-based therapies or cognitive enhancement. Impressively, preliminary studies have demonstrated that real-time fMRI feedback training can facilitate the control of chronic pain, tinnitus, emotion, and movement. Although these early demonstrations are encouraging, the regional BOLD feedback technique faces significant challenges - especially physiologic noise unrelated to the task. "Whole-brain" feedback approaches can help "cancel out" task-irrelevant noise (i.e., noise that is unrelated to two distinct alternating brain states), but the whole-brain approach lacks the regional specificity needed for therapeutics. Motivated by the need for regional specificity in therapeutics targeting particular brain regions, we have developed and tested a new real-time processing technique, Spatio-temporal Activity in Real-Time (STAR), that retains the robustness of the whole-brain approach (i.e. with suppression of noise), while providing spatial specificity.

Methods: BOLD fMRI at 3T (Siemens; TR = 2 sec) with a Partial Least Squares (PLS) linear classifier was used to characterize the whole-brain response for 19 subjects (12 male; 5 abstinent cocaine patients) during on-magnet instructions to alternate between two (30 sec each) sets of distinct thoughts: 1) repetitive motor (arm) activity - "Think about hitting a tennis ball (or similar activity)" vs. 2) spatial navigation - "Think about moving from room-to room in a familiar space". Following classifier training (approx. 4-6 minutes), subjects attempted to control a classifier-driven screen cursor (horizontal bars) solely with their thoughts. The STAR technique was evaluated, retrospectively, for five *a priori* regions of interest in these data (supplementary motor area, SMA; l. and r. parahippocampal place area, PPA; l. and r. retrosplenial cortex, RSC).

Results: Whole-brain classifier training was extremely rapid (~5 minutes) and robust: frame-by-frame classification accuracies during feedback ranged from .67-.93; $6 < t < 30$, $p < 10^{-6}$). Impressively, classifier-driven real-time fMRI feedback enabled each of the 19 participants to control the screen cursor solely with their thoughts.

The STAR technique was shown to provide significantly better (frame-by-frame) classification accuracy than conventional regional BOLD for all five *a priori* regions of interest. STAR outputs include static maps and dynamic (real-time) STAR movies.

Discussion: Real-time fMRI feedback with a whole-brain classifier enabled rapid control of a screen cursor solely by thought. STAR offers an appealing optimization for real-time fMRI applications requiring an anatomically-localized feedback signal. Though computationally demanding, the STAR optimization can be incorporated into standard real-time fMRI feedback protocols with conventional computing capabilities, facilitating feedback-based therapeutics. The speed, accuracy and ease of the approach - for both controls and cocaine patients - opens the way for wide-ranging real-time feedback studies in controls, and in clinical disorders characterized by compromised cognitive control, e.g., the addictions.

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148. This poster has been cancelled.

149. Neural Differences in Adolescents and Adults in Response to Monetary Anticipation

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Background: Adolescence is marked by coincidental increases in risk-taking behavior and psychiatric illnesses. Animal and human studies suggest that the reward system drives risk-taking behavior, though the neural mechanisms motivating risky behavior and its relationship to psychiatric illnesses remain unclear. The goal of this work is to examine developmental differences in functional magnetic resonance imaging (fMRI) activation patterns and functional connectivity between healthy adolescents ($n = 26$) and healthy adults ($n = 31$) using a well-accepted monetary incentive delay task (Knutson et al., 2001).

Methods: Subjects performed an event-related task in which a cue signaled that \$0.20, \$1 or \$5 could be won or lost if they responded fast enough to a visual target. fMRI preprocessing and analyses of the anticipatory phase was done with SPM8. Significance was set at $p < 0.005$, uncorrected.

Results: Each group was first examined separately. Overall, activation patterns of the reward system were similar across both groups.

In accordance with previous studies, elements of the reward system recruited by both adults and adolescents in response to monetary gain or loss anticipation included the striatum, dorsal anterior cingulate, insula, thalamus and brainstem. Preliminary results of a between-group, whole-brain analysis revealed a significant 2-way interaction for Group (adults vs. adolescents) x Valence (rewards vs. losses) in the right medial caudate. This interaction was driven by greater activation in adults than adolescents in anticipation of gain vs. loss. These results were further examined by conducting group analyses for each valence separately (gain, loss). During gain or loss anticipation, adults demonstrated greater activation in dorsal-lateral putamen than adolescents, and adolescents showed greater activation of the left pregenual cingulate cortex (BA 32/10), compared to adults.

Discussion: Our preliminary results suggest that, while adults and adolescents recruit similar regions during monetary incentive anticipation, differential recruitment of areas such as the putamen and anterior cingulate may account for developmental differences in sensitivity and response to reward anticipation. In addition, dynamic causal modeling in conjunction with known anatomic connections will be used to analyze the functional connectivity of *a priori* determined regions of interest: ventral striatum, ventral regions of pre-frontal cortex, thalamus and amygdala, and the influence of monetary reward anticipation on these regions and their functional correlations.

Ref: Knutson B, Adams CM, Fong GW, Hommer D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci.* 2001 Aug 15, 21(16) RC159 (1-5).

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150. Subacute and chronic regional metabolic changes with vagus nerve stimulation in treatment-resistant depression

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Background: Sustained stimulation of the left cervical vagus nerve (VNS) has been FDA-approved as an adjunct treatment for severe, treatment-resistant major depressive disorder (TRMD). Human and primate studies demonstrate the upstream afferent pathway of the vagus nerve intersects with multiple brain regions known to be involved with mood regulation (e.g., the amygdala, insular cortex, and orbitofrontal cortex). Existing neuroimaging studies demonstrate that the *acute* VNS is associated with changes in activity in multiple regions known to be associated with mood disorders, including the orbitofrontal cortex, anterior cingulate cortex, insular cortex, among other regions. Using ¹⁸Fluorodeoxyglucose (FDG) positron emission tomography (PET), this study assesses the effects of subacute (3 months) and chronic (12 months) VNS in 9 subjects with TRMD.

Methods: All subjects underwent careful screening to rule out other contributing factors to TRMD (e.g., personality disorder, substance abuse/dependence). Nine subjects (2 male, 7 female; average age 42 [sd = 10.9]) signed informed consent and underwent VNS implantation. Within two weeks of implantation, but prior to initiation of stimulation, each subject underwent baseline depression measures (Hamilton Depression Rating Scale, 24-item [HDRS-24 = primary measure], Montgomery Asberg Depression Scale [MADRS], and the Inventory of Depressive Symptoms-Self Report [IDS-SR]), and a 20 minute FDG PET scan (10 mCi ¹⁸FDG infused, followed by a 40 minute incubation). Subjects returned for repeat PET scans at 3 months and 12 months, at which time their devices were turned off for one hour prior to infusion of FDG and identical 20 minute PET scans. PET images were aligned, normalized for global uptake, and resampled to standard atlas space. Statistical t-images were calculated to evaluate VNS-induced changes occurring across time intervals (0-3 months; 0-12 months).

Results: Of the 9 subjects receiving 12 months of VNS, 7 responded (>50% drop in HDRS-24), and 3 remitted (HDRS-24 ≤ 9). Statistically significant ($p < .005$, whole brain uncorrected) increases in metabolically activity were observed in three regions: the left dorsolateral prefrontal cortex, left orbitofrontal cortex, and left anterior insular region. Preliminary correlations of regional metabolic activity demonstrated that baseline metabolic change in the bilateral anterior insular and orbitofrontal cortices correlated with degree of improvement in depression at 12 months ($r = .668-.886$; p range: .001-.049). Additionally, the amount of regional metabolic change in the first three months in multiple areas (orbitofrontal regions, dorsolateral prefrontal cortex, and anterior cingulate cortex) correlated strongly with change in depression between 3 and 12 months ($r = .665-.903$; p range: .001-.050).

Discussion: These findings are very preliminary and are based on a very small sample size. The changes observed do suggest that chronic VNS leads to increased metabolic activity in several regions known to be associated with mood regulation. Additionally, preliminary correlations suggest that early metabolic changes (0-3 months) correlate strongly with change in depression occurring between 3-12 months. This is consistent with the observation that most individuals receiving VNS for depression do not experience a change in depression for 6-7 months after initiation of stimulation. Further, these studies suggest the possibility that pre-treatment baseline metabolic function may allow some prediction of degree of anticipated improvement in depression with sustained VNS.

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151. Imaging Drug-Induced Dopamine Release in Rhesus Monkeys with [¹¹C]PHNO versus [¹¹C]raclopride PET

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Background: The radiotracer [¹¹C]PHNO may have advantages over other dopamine D₂/D₃ receptor ligands because as an agonist it measures only the high affinity - functionally active - D₂/D₃ receptors and not the low affinity receptors. Ginovart et al. (2006) demonstrated improved sensitivity over [¹¹C]raclopride for measuring amphetamine-induced changes in synaptic dopamine levels. Our aim was to take advantage of the strength of [¹¹C]PHNO for measuring the small dopamine signal induced by nicotine. Previously, Marengo et al. (2004) reported that a nicotine challenge (0.01-0.06 mg/kg, IV) yielded a 5% reduction and an amphetamine challenge (0.4 mg/kg, IV) in a 28% reduction in [¹¹C]raclopride binding potential in the caudate and putamen in monkeys. The goal of this study was to compare the sensitivity of [¹¹C]PHNO PET to that of [¹¹C]raclopride PET with nicotine- and amphetamine-induced dopamine release in nonhuman primates.

Methods: Four adult male rhesus monkeys were imaged on a FOCUS 220 PET scanner after injection of a bolus of [¹¹C]PHNO or [¹¹C]raclopride in 3 conditions: baseline; pre-injection of nicotine (0.1 mg/kg bolus + 0.07 mg/kg infusion over 30 min); pre-injection of amphetamine (0.4 mg/kg, 5 min prior to radiotracer injection). The mass dose of each radiotracer was held constant within each animal between scans. Dopamine release was measured as change in binding potential (BPND). BPND was estimated with simplified reference tissue model (SRTM) using the cerebellum as the reference region.

Results: With [¹¹C]PHNO, nicotine administration resulted in an average decrease of $10 \pm 8\%$ in BPND in the caudate and $11 \pm 8\%$ decrease in the putamen. Amphetamine administration resulted in a robust decrease of $48 \pm 4\%$ in the caudate and $49 \pm 12\%$ in the putamen. With [¹¹C]raclopride there was a nicotine-induced increase

in BPND of $3 \pm 1\%$ in the caudate and a decrease of $7 \pm 4\%$ in the putamen. Amphetamine administration resulted in a decrease of $36 \pm 14\%$ in the caudate and $34 \pm 14\%$ in the putamen.

Discussion: Our preliminary results do not support a substantive improvement in sensitivity to drug-induced dopamine release for [^{11}C]PHNO over [^{11}C]raclopride in the caudate and putamen. Nicotine-induced dopamine release measured with [^{11}C]raclopride is consistent with the findings by Marenco et al. (2004). Amphetamine-induced dopamine release measured with [^{11}C]PHNO was higher in this study compared to a study in humans ($0.38\text{--}0.45\text{ mg/kg}$ amphetamine, PO; Willeit et al., 2008), and comparable with Ginovart et al. (2006). We are currently investigating the utility of [^{11}C]PHNO in predominantly D3 regions and increasing our sample size.

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152. Diagnostic Accuracy of Biomarkers in Schizophrenia Compared by Means of Receiver Operating Characteristic Curves Will Cronenwett*, John Csernansky

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Background: Schizophrenia is a disabling disorder for which no clinically useful diagnostic test exists, although many candidate biological markers have been proposed. Receiver operating characteristic (ROC) curves are a useful way of comparing the relative utility of diagnostic predictor variables against one another when the test outcome is binary (e.g. presence or absence an illness). Furthermore, dividing a population based on the state of predictor variables creates groups with shared characteristics, and may suggest anatomical variants of the illness.

Methods: We acquired T1-weighted MR images from 110 people with DSM-IV-TR schizophrenia and from 110 healthy control subjects. We examined the shape (surface deformation) and volume of the thalamus, basal ganglia, hippocampus, and amygdala, which are structures known to be affected in schizophrenia. We collected data on 18 variables per subject. We randomly selected half of our sample to construct a test set, and then constructed ROC curves for each of our variables to identify the cut-point along each variable that best classified individuals in our test set according to the presence or absence of schizophrenia. By comparing the areas under the ROC curves, we were able to identify the best predictor overall. We used this marker to divide our test set into two groups and we then repeated the process, subdividing each group as far as we were able, continuing while groups contained enough individuals to enable the calculations, and as long as the results were statistically significant at $p < 0.01$. This allowed us to create a diagnostic decision tree in which each branching point represented a cut-point of a shape or volume variable, and each terminal node (or "leaf") represented a subset of our original sample that shared common shape and volume characteristics. We repeated this process using 9 more test sets constructed of different random selections of 50% of our total subjects, to create a total of 10 decision trees, which we then compared.

Results: In each of our 10 test sets, only two of the 18 variables tested were ever selected as first nodes of the decision trees. These were both measures of shape deformation (as opposed to volume). In six samples, the shape of the caudate was the best discriminator; in these, the nodes selected subsequent to the shape of the caudate (second level nodes) showed no discernable pattern. A more interesting pattern emerged in the other four decision trees. In these, the shape of the thalamus was the best predictor of schizophrenia. In each of these cases, one second level node was the shape of the caudate, indicating that shape changes in the caudate co-occurred with shape changes in the thalamus. In looking at all 10 decision trees, the first level node was always a measure of shape. Of the remaining branch points, 14 were

shape variables and only 4 were volumetric variables. The diagnostic accuracy of the best predictor variable ranged from 60.3% to 78.0%.

Discussion: The inconsistencies in our results suggest the need for larger sample size and more sensitive measures. Still, we have demonstrated that ROC methodology is useful for comparing the diagnostic accuracy of biological imaging markers in schizophrenia. In our sample, measures of shape deformation were consistently more reliable discriminators for the presence or absence of schizophrenia than volumetric measures, and the shape of the thalamus and caudate were the most useful overall. We present strong evidence that subjects with schizophrenia who have increased surface deformation of the thalamus also have co-occurring shape changes in the caudate. The caudate and thalamus are nodes along a thalamo-basal ganglia-cortical neural network; we may have identified a subgroup of people in whom pathological changes are distributed throughout this network.

Disclosure: W. Cronenwett: Part 1; Novartis Pharmaceuticals. J. Csernansky: Sanofi-Aventis, Eli Lilly.

153. Fronto-Limbic Connectivity in Adolescent Major Depression: Integrating Multi-Modal Imaging Techniques

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Background: The pathophysiology of major depressive disorder (MDD) involves fronto-limbic neural circuitry. Advanced magnetic resonance imaging (MRI) techniques to examine neural connections include diffusion tensor imaging (DTI), which measures microstructural integrity within white matter tracts ("structural connectivity"), and resting-state functional MRI (rsfMRI), which measures the correlation of brain activity between gray matter regions ("functional connectivity"). This research is particularly important in adolescence as neural connections are still undergoing development. Our previous work has identified altered fronto-limbic connectivity in adolescents with MDD, including (1) lower fractional anisotropy (FA) in the tract connecting right amygdala to subgenual anterior cingulate cortex (sgACC) [1], and (2) lower functional connectivity in a distributed fronto-limbic network stemming from sgACC [2]. The goal of the present study was to integrate these techniques to better understand the significance of altered connectivity in adolescent MDD. To do this, we re-analyzed our rsfMRI data to focus on the identical connection (sgACC-amygdala) in rsfMRI that we had examined with DTI.

Methods: Subjects: 14 adolescents with MDD aged 15-19 and 14 healthy comparison volunteers. (1) *Diffusion Tensor Imaging.* As previously reported [1], we used probabilistic tractography to delineate sgACC-amygdala tracts. Mean FA values within these tracts was measured for group comparison using t tests. (2) *Functional Connectivity.* We designed a new strategy to analyze our existing rsfMRI dataset [2] in order to focus in on the sgACC-amygdala tract. We extracted the time series of BOLD signal fluctuation in sgACC and bilateral amygdala, measured correlation between ROI timeseries and normalized the values to z scores for group comparison using t tests. (3) *Integration.* We used Pearson correlations to examine relationships between FA and rsfMRI z scores in the entire group and within subgroups.

Results: Group comparisons within both techniques revealed lower connectivity of sgACC-amygdala connections for the MDD group. For DTI, the finding was significant on the right ($p = 0.013$, Cohen's $d = 1.0$) and a trend on the left ($p = 0.13$, $d = 0.6$), while the rsfMRI finding was significant on the left ($p = 0.018$, $d = 0.7$) and not the right ($p = 0.24$, $d = 0.5$). Integration between imaging methods on connectivity of the sgACC-amygdala circuit was the primary focus of this study. For the whole sample, DTI and rsfMRI connectivity measures were significantly correlated on the left ($r = 0.546$, $p = 0.003$) and showed a trend correlation on the right ($r = 0.350$, $p = 0.08$). For controls, the correlation between measures was a trend on the left ($r = 0.465$, $p = 0.094$) and significant on the right ($r = 0.618$, $p = 0.024$). The MDD

group showed a trend-level correlation ($r = 0.518$, $p = 0.07$) on the left but no significance on the right ($r = 0.053$, $p = 0.351$). To further examine the divergence on the right, a Fisher r - z transformation to test the significance of the difference of correlation coefficients between groups revealed a trend level of significance ($p = 0.06$).

Discussion: We report integrative results using multi-method imaging approaches to examine brain connectivity within a key neural circuit in adolescents with MDD. In order to best link the two methods, we re-analyzed our resting-state fMRI data to examine the identical connection (amygdala to sgACC) as the DTI analysis. Both techniques revealed lower connectivity in this circuit in the MDD group, but the findings were significant for DTI on the right and rsfMRI on the left. Our integrative analyses revealed that the connectivity measures were correlated in the group as a whole and in the control group, but were divergent on the right for the MDD group. A developmental hypothesis to explain this divergence is that adolescents early in the course of depression demonstrate aberrant development of white matter tracts, and as an adaptive mechanism may develop aberrant functional connectivity patterns. Future multi-modal research with larger samples and a longitudinal component will be required to further investigate the neurodevelopmental underpinnings of adolescent MDD.

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154. Attention and Affect in Bipolar Disorder: An fMRI Study

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Background: Bipolar disorder is characterized by recurrent depressive and/or manic mood episodes that interfere with psychosocial functioning. A prominent feature of bipolar disorder is cognitive impairment, which contributes to impairments in psychosocial functioning. There is little information known about the functional neuroanatomy that underlies concentration and memory difficulties associated with depressed mood in bipolar disorder. This study examined the impact of negative affect on neural networks involved in attention in patients with bipolar disorder with depressive symptoms.

Methods: 40 individuals with DSM-IV bipolar-I disorder (BP-I, 23 females, HAMD: $M = 12.2$, $SD = 6.6$; YMRS: $M = 4.8$, $SD = 5.1$) and 34 healthy control participants (15 females; HAMD: $M = 1.0$; $SD = 1.4$; YMRS: $M = 0.4$; $SD = 0.9$) matched for age, and education (all right-handed) completed an affective attention task while undergoing functional Magnetic Resonance Imaging (fMRI). MRI data were acquired using a 3.0-T whole-body scanner (Trio-System), equipped for echo planar imaging (Siemens Medical Systems, Iselin NJ) with a 3-axis gradient head coil. In the fMRI paradigm, subjects were shown three-digit numbers (100, 020 or 003). The task was to decide which number was different from the two other numbers (e.g. 1 0 0 - correct answer = 1; 0 2 0 - correct answer = 2). The numbers were superimposed on neutral, negative or positive affectively valenced pictures taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2005). We predicted that individuals with bipolar disorder would show abnormal activations in affect relevant areas (e.g., amygdala, insula, medial, ventral and dorsal prefrontal cortex,) as well as areas involved attentional control (e.g., anterior cingulate [ACC] and dorsolateral prefrontal cortex [DLPFC]) in particular for negatively valenced pictures. Data were analyzed with SPM 5 using a random effects model.

Results: There were no group differences in amygdala activations for any of the comparisons between BP-I and control participants.

When BP-I participants completed the attention task during negatively valenced IAPS pictures relative to neutral IAPS pictures, they exhibited an activation increase in the anterior insula, pre-genual ACC (BA 32; affective division of the ACC), dorsal ACC (BA 32; cognitive division of the ACC), the dorsomedial prefrontal cortex (BA 10), the lateral ventral prefrontal cortex (BA 11/47) and the DLPFC (BA 9/46) compared to control participants. For negatively valenced pictures compared to positively valenced pictures, BP-I participants exhibited increased activation in those regions that were observed for the negatively and neutrally valenced pictures. This included the anterior insula, pre-genual (BA 32 and dorsal ACC (BA 32), dorsomedial PFC (BA 10) and DLPFC (BA 9/46).

Discussion: To our knowledge this is the first study that investigates the functional neuroanatomy of task irrelevant affect during concurrent completion of a cognitive task. There was a striking absence of increased amygdala activation for bipolar disorder participants compared to control participants. Amygdala activations have been consistently reported in previous studies using affective stimuli without a concurrent cognitive task in bipolar disorder (e.g. Lawrence et al., 2004). For BP-I participants compared to control participants we observed increased activations in prefrontal regions involved in the regulation of emotional responses. This may reflect increased demands on compensatory emotion regulation processes in BP-I and/or dysfunctions in prefrontal cortical regions involved in emotion regulation in BP-I.

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155. Amygdala Connectivity in Youth with a Bipolar Parent

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Background: Child and adolescent offspring of parents with bipolar disorder have an elevated risk of developing bipolar disorder but typically have not yet developed the illness. Therefore, young offspring of parents with bipolar disorder ("at-risk", AR) is an ideal population in which to identify prodromal clinical and neurobiological manifestations of incipient mania. Recent findings suggest that adolescents with bipolar disorder exhibit structural and functional abnormalities in the amygdala, however, whether these abnormalities are present prior to illness onset remains unclear. Moreover, several studies suggest that AR youth exhibit elevated rates of mood and behavioral disorders. However, to our knowledge, few studies have examined amygdala dysfunction in AR youth and its association with psychopathology, which may clarify the neurodevelopmental course of bipolar disorder. With these considerations, we hypothesized that AR youth would exhibit abnormalities in amygdala connectivity with other brain regions. Additionally, we hypothesized that compared with healthy youth without a bipolar parent, AR youth with attention-deficit hyperactivity disorder would exhibit greater abnormalities in amygdala-prefrontal connectivity than AR youth without ADHD.

Methods: Fifty youth (ages 10-20 years) without bipolar disorder and with a bipolar parent and 20 healthy subjects without a first- or second-degree relative with a mood or psychotic disorder were recruited. All subjects and their legal guardians provided assent and consent, respectively. Additionally, parents were evaluated using the Structured Clinical Interview for DSM-IV (SCID-P/L) and child and adolescents study participants were assessed using the Washington University Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U K-SADS) administered by raters with established symptom and diagnostic reliability ($\kappa > 0.9$). All participants underwent functional MRI scans while performing a Continuous Performance Test with Emotional and Neutral Distracters (CPT-END). Scans were performed at the University of Cincinnati College Of Medicine Center for Imaging Research using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system. This CPT-END task is a visual oddball paradigm with the addition of emotional and neutral pictures taken from the International Affective Picture System (IAPS). A functional connectivity analysis was performed using the left and right amygdala as seed regions. Functional images were analyzed using Analysis of Functional Images (AFNI) and included motion correction, spatial smoothing, normalization and random effects analysis of activation data. Voxel-wise functional connectivity analyses were then performed using the left and right amygdala as seed regions.

Results: At-risk youth exhibit increased association between right amygdala and right Brodmann area (BA) 11, right amygdala and BA 24, and left amygdala and left medial temporal gyrus compared with healthy youth during CPT-END performance ($p < 0.05$). The most common psychiatric disorder in our AR sample is ADHD ($n = 11$, 22%). Compared with the AR without ADHD group, greater connectivity between left amygdala and left BA 10 is present in the AR with ADHD group (vs. healthy comparison group). During target stimuli, the AR with ADHD group exhibits greater correlation between left amygdala and left BA11 ($r = 0.75$), compared with the healthy ($r < -0.21$) and AR without ADHD ($r = 0.03$) groups ($p < 0.05$). However, during emotional distracter stimuli the AR with ADHD ($r = 0.36$) and healthy ($r = 0.66$) groups exhibit greater correlations between left amygdala and left BA 11 compared with the AR without ADHD group ($r = -0.10$, $p < 0.05$).

Discussion: Our preliminary findings indicate that youth at risk for developing bipolar disorder exhibit alterations in prefrontal-amygdala coupling. Specifically, AR youth, particularly those with ADHD, do not exhibit the healthy reciprocal relationship between BA 11 and the amygdala during tasks of attention. In contrast, during emotional

stimuli, AR youth without ADHD do not exhibit normal coupling between these regions, suggesting that symptoms of inattention may be compensatory for early neurobiological changes associated with bipolar disorder. Future longitudinal neuroimaging studies of youth with a familial risk of bipolar disorder are needed to confirm these findings.

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156. Altered Language Network Activity in First-Degree Relatives of Persons with Schizophrenia

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Background: Language abnormalities and thought disturbances are core features of schizophrenia (SZ). Structural and functional abnormalities in frontal and temporal brain regions subserving language perception and processing are observed in SZ.¹ Our preliminary work demonstrates similar abnormalities in first-degree relatives of persons with SZ, who are at familial high risk for the disorder (HRSZ)²⁻⁴. Here, we extend this work, using an event-related semantic priming task^{1,5} to examine how the semantic relationship of prime and target words modulates hemodynamic activity during lexico-semantic processing in young adult HRSZ compared to healthy controls.

Methods: Subjects were 22 non-psychotic HRSZ and 21 controls comparable in age (range: 19-32), sex, ethnicity, and handedness. All subjects had IQ > 85 and were free of current psychoactive medication or lifetime history of psychotic disorder. Task-related hemodynamic response (measured as blood oxygen level-dependent signal) was estimated using functional magnetic resonance imaging (fMRI) scans collected at the Massachusetts Institute of Technology on a Siemens 3.0 Tesla scanner using a 32 channel head coil. Mood was assessed just prior to scanning using the Profile of Mood States (POMS). During fMRI, subjects viewed directly related, indirectly related, and unrelated prime-target word-pairs as they performed a lexical decision task, in which they decided whether each target was a real word or a nonword^{1,5}. Hit rate and reaction time (RT) were dependent variables. fMRI Analyses: fMRI data were analyzed in SPM8. A canonical SPM hemodynamic response function was used to estimate the hemodynamic responses for the conditions of interest. For each subject, contrasts for the 1) directly-related > unrelated and 2) indirectly-related > unrelated conditions were generated and submitted to second level, random-effects analyses. Between-group differences were tested using two-group t-tests or ANCOVA (using parental education level as a covariate), and were corrected for multiple comparisons 1) across the whole-brain, or 2) using *a priori*, task-specific anatomically-defined regions of interest (ROIs) created using the Wakeforest University Pickatlas Tool (corrected using a family-wise error correction with a cluster-level significance of $p < .05$). ROIs were the left anterior inferior prefrontal gyrus (BA 45, 47), bilateral orbitofrontal gyrus (BA 10,11) and anterior cingulate (BA 24/32), the left lateral temporal cortex (including the superior temporal gyrus and sulcus and middle temporal gyrus; BA 22, 42, 21)1, bilateral temporal fusiform cortex (BA 37, 20)1, and the left hippocampus1 and parahippocampus.

Results: While the controls had a higher level of parental education (an estimate of socioeconomic status), there were no group differences in other demographic or neuropsychological variables, or in mood state on the day of scanning. In addition, there were no group differences in semantic priming (replicating previous findings in SZ using this task). As expected, semantic relationship modulated the location of hemodynamic response signal. During lexical processing of directly related (relative to unrelated) word-pairs, HRSZ showed 1) less activation (compared to controls) in the left superior frontal gyrus and inferior temporal cortex (BA 38), and 2) greater activation in the left posterior hippocampus and parahippocampus. This was because HRSZ failed to activate left inferior temporal regions, suppressed activity in frontal regions (where controls activated), and enhanced activity (relative to controls) in the hippocampus and parahippocampus. During lexical processing of indirectly related (relative to unrelated) word-pairs, HRSZ showed less activation than controls in clusters which included 1) the left inferior parietal lobule, the middle and superior temporal gyrus (BA 40/39/22), 2) the left inferior and middle temporal gyrus (BA 20/21), 3) left temporal pole and 4) the left insula and inferior frontal gyrus (BA 13/44/45/47)(all clusters corrected across the whole-brain). These differences were due to HRSZ suppressing activation in the same regions in which controls showed significant activation.

Discussion: Familial risk for SZ may be associated with failure of left frontal- and temporal- activation (and hippocampal hyperactivity) in response to semantic associations. Additional analyses will examine relationships between these functional changes and white matter abnormalities in HRSZ. Future studies should examine whether these potential endophenotypes can predict which individuals go on to develop SZ.

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157. Neural striatal correlates of locomotor activity in typical children and adults

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Background: Although hyperactivity is a cardinal symptom of Attention-Deficit/Hyperactivity Disorder (ADHD), the presence of the symptom is dependent on subjective judgments. Recently, micromovements measured with an infrared camera have been shown to robustly distinguish patients with ADHD from healthy controls and to accurately predict treatment response outcomes. Here, we aim to build a base for future studies linking objective behavioral markers in ADHD and other psychiatric conditions to neural networks across age groups. Specifically, we aimed to construct a template of the neural correlates of micromovements in neurotypical individuals using fMRI-based resting state functional connectivity (RSFC) measures. Secondly, we examined the extent to which the neural correlates of micromovements differ between child and adult populations.

Methods: Forty-four healthy participants, 20 children and 24 adults (ages 7-12 and 18-48, respectively) participated in this study and signed informed consent and assent forms as approved by the NYU School of Medicine IRB. An infrared motion tracking system quantified micromovements while each participant performed a continuous performance task (Quotient™ ADHD System). A 6.5 minute resting state scan was collected for each participant. Following standard image preprocessing, nuisance signals were removed and the residual mean time-series for each voxel was extracted. Given that striatal circuitry is heavily implicated in motor function, we selected striatal seeds validated by our previous work (Di Martino et al., 2008). Caudate seeds were placed in the dorsal caudate (DC), the inferior ventral

striatum, and the superior ventral striatum. Putamen seeds were located in the ventral-rostral putamen, the dorsal-caudal putamen, and the dorsal-rostral putamen. For each seed, we computed voxel-wise correlations for each participant. Within- and between-group analyses were carried out using random-effects models. Micromovements for the child and adult groups were modeled using independent predictors. These steps generated maps of regions exhibiting significant positive and negative RSFC related to movement within each group, and maps with significant RSFC differences between groups for each seed region (Gaussian random field theory corrected; $Z > 2.3$; $p < 0.05$).

Results: Group analyses showed significant relationships between micromovements and RSFC of putamen and caudate seeds. Some of these relationships were shared between both adult and child groups. We found a positive relationship between micromovements and RSFC of the left putamen seeds with unimodal and heteromodal sensory processing regions. These included temporal-occipital fusiform gyrus bilaterally, and left cuneus. These regions are not typically considered to be part of putamen functional circuitry. Greater movement was also related to increased RSFC between right DC with right pre- and postcentral gyri, again areas not included in DC circuitry. These findings of a positive relationship between micromovements and RSFC in areas not typically related to the regions of interest - i.e, ectopic RSFC - suggest that poor segregation of motor and cognitive striatal-based circuits is associated with greater locomotor activity. Additionally, we found age-dependent relationships. Relative to children, adults had stronger correlations between motor activity and putamen-based RSFC with sensory processing areas including the left fusiform, lingual gyri and lateral occipital cortex. On the contrary, a greater relationship with micromovements and RSFC of the dorsal putamen with pregenual anterior cingulate cortex, typically included in the default network, was evident in children relative to adults.

Discussion: Our analyses suggest a relationship between objective markers of locomotor activity and the organization of striatal functional circuitry in healthy controls, demonstrating the presence of shared and unique neural correlates for micromovements in child and adult populations. This approach appears to also have the potential to reveal relevant group differences in neural circuit in ADHD and other psychiatric disorders characterized by locomotor hyperactivity.

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158. Changes in Glucose Metabolism with Antidepressant Response to Ketamine in Treatment-Resistant Depression

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Background: Multiple lines of evidence support the role of the glutamatergic system in the pathophysiology and treatment of major depressive disorder (MDD). Consistent with this hypothesis, ketamine, an N-methyl D-aspartate (NMDA) antagonist, has been shown to induce a rapid and significant reduction in depressive symptoms in subjects with treatment-resistant depression [1]. The neural mechanisms underlying this effect are unknown. We used [18F]fluorodeoxyglucose (FDG) positron emission tomography (PET) to examine the cerebral metabolic response to ketamine infusion in subjects with treatment-resistant MDD. Because the FDG PET measure of glucose metabolism is dominated by the uptake of glucose into glia in response to neuronal glutamate release, we expect brain metabolism to accurately reflect the influence of ketamine on glutamate transmission. **Methods:** Twenty subjects with treatment-resistant MDD (unmedicated > 2 weeks) underwent PET scanning at baseline and 120 minutes after a single dose of ketamine (0.5 mg/kg infused IV over 40 min).

PET scans were acquired on a GE Advance scanner after injection of 4.5 mCi of FDG. Structural MRI scans were performed for anatomic localization. Regional cerebral metabolic rate for glucose (rCMRGlu) was calculated using an image-derived cardiac input function and venous blood glucose samples (due to failure of the input function measurement, rCMRGlu was not calculated in 1 subject). Mean rCMRGlu values were derived from pre-determined regions of interest placed on individual subject MRIs and masked with a map of gray matter. Mean values were normalized by whole brain metabolism. The Montgomery-Asberg depression rating scale (MADRS) was administered at baseline and 230 minutes post-ketamine infusion, and the percent change in score was correlated with the change in rCMRGlu. A post-hoc voxel-wise whole brain analysis of raw radioactivity images ($N=20$) was performed in SPM5. For the SPM results, coordinates are given in MNI space, and p -values are corrected for multiple comparisons.

Results: Whole brain metabolism did not differ between the baseline and ketamine conditions ($p=0.341$). Compared to baseline, rCMRGlu following ketamine infusion was increased in the posterior cingulate ($p=0.019$) and superior temporal gyrus (STG; $p=0.013$). The change in rCMRGlu was negatively correlated with the percent change in MADRS scores in the right superior temporal gyrus ($R=-0.583$, $p=0.009$), and positively correlated with the percent change in MADRS scores in the right hippocampus ($R=0.457$, $p=0.049$). The voxel-wise analysis showed an increase in FDG uptake following ketamine infusion in the right inferior occipital gyrus (34, -88, -12; 4697 voxels, $p=0.001$) and the left parahippocampal gyrus (-22, -48, -10; 3274 voxels, $p=0.004$). Both clusters appeared to be bilateral, although only one side reached significance. A decrease in FDG uptake following ketamine infusion was found in the right insula (40, 6, 14; 2869 voxels, $p=0.008$).

Discussion: Ketamine infusion increased metabolism in the posterior cingulate, STG, occipital cortex, and parahippocampal gyrus relative to baseline. In the STG, this increase in metabolism was significantly associated with the reduction in depression severity ratings. Anatomical studies have shown that the posterior cingulate, STG, and parahippocampal cortex share extensive interconnections with the medial prefrontal cortex to form part of an extended "visceromotor network" that modulates autonomic, endocrine, behavioral and experiential aspects of emotional behavior. Reduced metabolism in the STG has been shown to correlate with psychomotor-anhedonia aspects of depression [2], while previous studies have demonstrated increases in posterior cingulate metabolism during remission from MDD [3]. Ketamine infusion also appeared to decrease metabolism in the insula, consistent with evidence that baseline metabolism is elevated in the insula in MDD [4], and that metabolism increases in putative rodent analogues of depressive behavior. Elucidation of the neurobiological effects ketamine infusion may result in a greater understanding of rapid antidepressant response and guide future development of novel treatments for MDD. [1] Zarate Jr., CA, et al. Arch Gen Psychiatry 63(8):856-64 (2006). [2] Dunn, RT et al., Biol Psychiatry 51(5):387099 (2002). [3] Mayberg, HS et al., Am J Psychiatry 156(5):675-682 (1999). [4] Drevets, WC et al., Current Opinion in Neurobiology 11(2):240-249 (2001).

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159. The Role of Norepinephrine Transporter in Attention Deficit Hyperactivity Disorder

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Background: Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent psychiatric disorder in children that continues into adulthood; however, its pathogenesis is poorly understood. It is widely accepted that dysfunction in DA and NE circuits underlies the cognitive and behavioral impairments characteristic of ADHD. We have previously shown that methylphenidate, the most commonly used drug for treatment of ADHD, significantly occupies the norepinephrine transporter (NET) at clinically relevant doses with an ED₅₀ even more potent than that for the dopamine transporter (0.14 and 0.25 mg/kg for NET and DAT, respectively), suggesting an important role for NET in ADHD. The purpose of this study is to determine whether adults with ADHD have altered NET availability compared to matched controls (HC) as measured by the ligand (S,S)-[¹¹C]MRB ([¹¹C]MRB) and PET.

Methods: Adults who met DSM-IV criteria for ADHD were recruited ($n=5$). Their binding potential (BPND) values were compared with the mean BPND values obtained from HCs ($n=23$). Subjects underwent dynamic PET acquisition using HRRT for 120 min following a bolus injection of ~ 740 MBq of [¹¹C]MRB. BPND images were computed using the multilinear reference tissue model (2-parameter version: MRTM2 with $t^*=20$) with occipital cortex as the reference region. The mean BPND values were estimated for 11 ROIs (including small brain regions; i.e., locus coeruleus (LC), brainstem nuclei, hypothalamus (hypoTH), and thalamic subnuclei) and compared between the two groups using two-tailed unpaired t -test.

Results: In two subjects with ADHD (ages 40 and 49) who had not used stimulants for over 10 years, a decrease in BPND values was observed in most NET-rich regions; specifically in thalamus (-28%), LC (-24%), hypoTH (-61%), and thalamic subnuclei: dorsomedial (-36%) and pulvinar (-42%). The highest decrease in BPND was observed in nucleus ruber (-63%). Interestingly, in a third ADHD subject (age 27, who used cocaine between age 21-25), the trend of decreased BPND was still observed [e.g., thalamus (-13%), hypoTH (-40%), and thalamic subnuclei: dorsomedial (-20%) and pulvinar (-23%), with the highest decrease still in nucleus ruber (-62%)] however, higher BPND values in the NET-rich regions were noticed in this subject when compared to the other two ADHD subjects. Based on our previous finding that NET is decreased with age and up-regulated with cocaine use (Ding et al., Synapse, 2010), higher BPND values in this ADHD subject could be explained by the younger age and cocaine use. Detailed results for all subjects will be presented.

Discussion: These preliminary data show a trend for altered NET in ADHD subjects, with a down-regulation in most NET-rich regions. These results are also consistent with our previous finding that NET is decreased with age and up-regulated with stimulant use. More ADHD subjects are under investigation and a careful statistical analysis will be required to confirm these encouraging results. Support: NIDA, CTSA from the National Center for Research Resources, a component of the National Institutes of Health (NIH), and NIH roadmap for Medical Research.

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160. Decreased Fronto-striatal Structural Connectivity in ADHD

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Background: Fronto-striatal regions have been implicated in the neurobiology of ADHD, but little work has addressed the white matter connections between them directly. The present study investigates the microstructural organization and myelination of fronto-striatal white matter in children with ADHD and controls.

Method: Diffusion Tensor Imaging (DTI) and Magnetization Transfer Imaging (MTI) imaging scans were acquired from 30 children with ADHD and 34 controls. A study-specific volume of interest (VOI) of fronto-striatal white matter was created using a tractography-based statistical group map. Fractional anisotropy (FA) is a measure of directional diffusion derived from DTI and indexes microstructural organization. The magnetization transfer ratio (MTR) is derived from MTI and indexes myelination more directly. Both measures were computed for the fronto-striatal VOI and for total cerebral white matter.

Results: Fronto-striatal FA but not MTR was decreased in ADHD. There were no differences in FA or MTR for total cerebral white matter. Fronto-striatal FA correlated negatively with teacher-rated attention problems in controls, but not children with ADHD.

Discussion: Changes in fronto-striatal connectivity in ADHD appear to be related to changes in microstructural organization rather than myelination *per se*. A correlation with attention problems for controls but not children with ADHD, suggest that fronto-striatal organization is relevant to ADHD symptoms, but that additional neurobiological factors may be involved for children with ADHD.

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161. Brain-Derived Neurotrophic Factor (BDNF) Val⁶⁶Met Polymorphism Affects Resting Hippocampal Activity in Medication-Free Patients with Schizophrenia

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Background: The Val⁶⁶Met single nucleotide polymorphism (SNP) in the Brain-Derived Neurotrophic Factor (BDNF) gene impairs activity-dependent BDNF release in cultured hippocampal neurons. In healthy humans, this SNP predicts worse episodic memory, reduced hippocampal volume and neuronal integrity, and abnormal task-related hippocampal activation¹. A role for BDNF in the pathophysiology of schizophrenia is supported by reports of abnormal expression² and transcription³ of BDNF in schizophrenic postmortem brain tissue and associations in patients between the Val⁶⁶Met SNP and age of illness onset⁴, negative symptoms⁵, aggressive behavior⁶, and exaggerated hippocampal volumetric changes⁷. Though data are mixed, BDNF genetic variation including this SNP confers schizophrenia risk in some cohorts⁸. Along with multi-modal evidence for hippocampal dysfunction in schizophrenia⁹, these data indirectly suggest a meaningful relationship between this illness and genetically-determined BDNF function in the hippocampus, though direct support is lacking. Here, we build on our previous work identifying effects of the Val⁶⁶Met SNP on resting hippocampal regional cerebral blood flow (rCBF) in healthy individuals to determine whether patients show abnormalities of this genotype-neurophysiological association.

Methods: Participants were 47 patients with schizophrenia or schizoaffective disorder (10 non-Caucasian, mean age 28 ± 6, 13 female) and 64 comparison individuals with no confounding medical, psychiatric, or substance use disorder by clinician-acquired history and physical examination (2 non-Caucasian, mean age 29 ± 7, 21

female). Each subject was genotyped for the Val⁶⁶Met SNP via TaqMan assay and underwent ¹⁵O-H₂O positron emission tomography (10 mCi/scan, two scans each) to measure resting rCBF. Patients were medication-free for 4 weeks prior to scanning according to a double-blind, placebo-controlled standard antipsychotic treatment withdrawal protocol. After attenuation correction and reconstruction, acquired volumes for each subject were background activity corrected, registered, spatially normalized to standardized space, smoothed, scaled and averaged using SPM5 (Wellcome Department of Cognitive Neurology, London, England). Average resting rCBF across a bilateral hippocampal region of interest (ROI) defined with PickAtlas software (Wake Forest University, Winston-Salem, NC) was used for subsequent general linear model analyses.

Results: There were no significant differences in age or sex distribution across genotypes and groups. Among healthy individuals (44 Val/Val, 20 Met carrier), harboring a Met allele was associated with significantly lower average hippocampal rCBF, whereas patients (32 Val/Val, 15 Met carrier) showed the opposite relationship, resulting in a significant group-by-genotype interaction ($p = 0.001$). Confirmatory voxel-wise comparisons within the ROI localized this finding to bilateral anterior and left posterior hippocampi ($p < 0.05$, FDR corrected). This interaction remained significant after reanalysis with only Caucasian participant data. Exploratory analyses of hippocampal-prefrontal covariation also revealed a group-by-genotype interaction ($p < 0.001$, uncorrected).

Discussion: These data suggest the possibility that task-independent hippocampal neurophysiology responds to the challenge of Met allele-mediated relative deficiencies in BDNF secretion in a divergent manner when occurring in the context of schizophrenia. Potentially consistent with the hypothesis that cellular sequelae of the BDNF Val⁶⁶Met SNP interface with aspects of schizophrenic hippocampal and frontotemporal dysfunction, this result warrants future investigation to understand relative contributions of unique patient trait (e.g., abnormal hippocampal neurodevelopment, neuroleptic exposure, chronic stress) or state (e.g., unmedicated psychosis) variables to this robust interaction.

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162. Corticolimbic Processing of Pain in Posttraumatic Stress Disorder

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Background: Epidemiological data suggest that posttraumatic stress disorder (PTSD) may be associated with sensitization to pain.

Methods: To pursue this issue, we administered noxious thermal skin stimuli during functional magnetic resonance imaging to subjects with chronic PTSD and to mentally healthy individuals ($n = 12$, each group). Neuroimaging data were also collected for aversive and pleasant images from the International Affective Picture System (IAPS).

Results: PTSD and healthy subjects provided similar ratings of both thermal sensory thresholds and of the temperatures. When 46 °C and 44 °C were contrasted to 42 °C, voxelwise between-group comparison

revealed smaller left amygdala activation in PTSD subjects along with greater bilateral striatal, thalamic, hippocampal and medial prefrontal and orbitofrontal cortices activations. Analyses of IAPS responses revealed decreased activation in the PTSD patients that included bilateral amygdala and thalamus for the aversive images and the orbitofrontal, cingulate and parietal cortices in conjunction with dorsal striatum for the pleasant images.

Discussion: These findings suggest that pain sensitization in PTSD: 1) involves other neural pathways than those mediating subjective pain perception and 2) that it is specific to the noxious thermal sensations and does not generalize to negatively- and positively valenced psychosocial stimuli.

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163. Effects of lithium on the pattern of cerebral activation during a verbal episodic memory task in patients with bipolar disorder

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Background: Studies in bipolar patients have shown diffuse cognitive changes that can persist during periods of euthymia although the origin of this deterioration hasn't been fully elucidated. (1,2) Previous studies from the Psychiatry Research Group at the University of Antioquia, Medellín (Grupo de Investigación en Psiquiatría - GIPSI) have shown reliable neuropsychological differences in episodic memory (3,4) indicating that these tasks may be a useful paradigm in which to investigate cognitive change in bipolar disorder using function imaging.

Methods: The study compared five euthymic patients diagnosed with Bipolar Disorder I who were being treated with lithium monotherapy, four euthymic patients diagnosed with Bipolar Disorder I who were not taking any medication, and five healthy controls without medication. Participants were presented with word pairs in the fMRI scanner in blocks, some with a semantic association and some without. Each block was followed by a single probe recognition test for one of the word pairs. Reaction times and changes in BOLD signal intensity were analysed between blocks to examine the effect of semantic association on memory. A Bayesian mixed effects model was also applied in FSL as a higher level analysis of the BOLD data to generate maps of activation to uncover group neural activation effects. The Talairach Client version 2.4.2 was used for the localisation and demarcation of cerebral activation.

Results: Using a threshold of a z score greater of equal to 2.3, adjusted for confounding variables, similar patterns of activation were found between patients without medication and healthy controls - which included activation in frontal and occipital areas arising from visual perception and executive control during the task. However, in patients being treated with lithium there was additional activation in the parietal cortices.

Discussion: Patients with Bipolar Disorder I under treatment with lithium needed to involve a great number of areas to complete the episodic memory with semantic association task. However, owing to the small number of participants in each group, the explanation for this difference needs to be proposed with caution and the paradigm needs to be extended to a greater number of patients to examine this effect further. However, one explanation may be that lithium generates changes in the extent of activation in cerebral area linked to task completion. We hope that this form of paradigm has the potential to clarify the neurocognitive effect of medication in patients with Bipolar Disorder.

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164. Beta₂-nAChR Receptor Availability Is Lower in Recently Abstinent Smokers with Schizophrenia Compared to Healthy Smokers

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Background: Individuals with schizophrenia have among the highest rates of tobacco smoking. The effects of nicotine are mediated by the nicotinic acetylcholine receptors (nAChRs), predominantly those containing the beta₂ subunit. Chronic nicotine exposure upregulates nicotinic agonist binding to brain beta₂ nicotinic acetylcholine receptors (nAChR). Postmortem studies have shown that there are region-specific increases in high affinity nAChR binding in healthy human tobacco smokers. However, postmortem studies suggest that smokers with schizophrenia, lack region-specific increases in high affinity nAChR binding. Tobacco smoke and nicotine have been shown to alleviate some of the cognitive deficits associated with schizophrenia, specifically on tasks of visual attention and inhibition. Based on these findings, we hypothesized that the beta₂-nAChR availability will be lower in smokers with schizophrenia versus age- and sex-matched healthy smokers in the frontal, parietal, and occipital cortices. We also hypothesized we would observe smoking abstinence-associated cognitive deficits in schizophrenic subjects.

Methods: To date, eleven men smokers with schizophrenia (41 ± 13yo) and eleven age and sex-matched control smokers (40 ± 12yo) participated in one MRI and one [I-123]5-IA-85380 SPECT imaging study after 5-9 days of smoking abstinence.

Results: Patients reported slightly greater nicotine dependence (FTND; 6 ± 2 vs. 4 ± 3) and smoked a greater number of cigarettes/day than controls (22 ± 2 vs. 17 ± 2) at intake. On the SPECT scan day, craving for cigarettes was significantly greater for patients vs. controls. Preliminary data indicate significantly lower β₂-nAChR availability in smokers with schizophrenia as compared to healthy smokers in the parietal (21%, $F = 4.4$; $p = .05$) and frontal (26%, $F = 8.8$, $p = .01$) cortices and thalamus (21%, $F = 5.0$, $p = .04$); with a trend toward significance in the anterior cingulate (18%, $F = 3.7$, $p = .07$), temporal (18%, $F = 3.7$, $p = .07$), and occipital (19%, $F = 4.1$, $p = .06$) cortices. We also observed that different aspects of cognition are regulated differentially by tobacco smoke: there was a significant decline in verbal memory (immediate recall $F = 7.1$, $p = .01$; delayed recall $F = 7.6$, $p = .01$) but not processing speed after tobacco withdrawal. However, after smoking resumption processing speed ($F = 7.4$, $p = .01$) and delayed recall ($F = 9.2$, $p = .004$) improved as compared to the smoking abstinence period.

Discussion: Overall, these preliminary findings may reflect a failure to upregulate beta₂-nAChR in schizophrenic smokers and provide further evidence for the cognitive benefits achieved from tobacco smoking in this population.

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165. Common abnormalities and disorder-specific compensation during implicit regulation of emotional processing in anxiety versus depression

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Background: Anxiety and depressive disorders are both associated with abnormalities in the processing and regulation of emotion. Despite this, little is known about the similarities and differences between anxiety and depression at the neural level. Such research is essential, however, for understanding the organization and structure of mental illness, informing ideas about vulnerability, and defining opportunities for intervention.

Methods: 32 healthy controls, 18 Generalized Anxiety Disorder (GAD) only patients, 14 Major Depression (MDD) only patients, and 25 comorbid GAD/MDD patients were studied using functional magnetic resonance imaging while they performed an emotional conflict task, which involved categorizing facial affect while ignoring overlaid affect label words. We compared trial-by-trial changes in conflict regulation, a test of implicit regulation of emotional processing, using behavioral and neural measures.

Results: Implicit regulation of emotional conflict, indexed with reaction times, was abnormal only in patients with anxiety (i.e. GAD or comorbid GAD/MDD). By contrast, activation of the ventral anterior cingulate and amygdala - areas previously implicated in regulating emotional conflict - was abnormal in all patient groups. Only MDD patients, however, were able to compensate for this deficit by also activating bilateral anterior lateral prefrontal cortices, wherein activity correlated with reaction times.

Discussion: These results point to important neurobiological differences between anxiety and depression, along with key similarities, and suggest novel avenues for understanding these disorders. Specifically, these data support the existence of a common abnormality in ventral cingulate-amygdalar regions, which may relate to a shared genetic etiology. Compensatory engagement of cognitive control circuitry in MDD, which accounts for the disorder-specificity of the behavioral phenotype, illustrates how the complex nature of psychopathology arises from the interaction of deficits and compensation, all of which can occur at an implicit level. Finally, these data highlight the importance of behavioral deficits for the interpretation of neuroimaging findings, and the unique utility of studying multiple patient cohorts simultaneously.

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166. Visual Processing in Anorexia Nervosa and Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) and anorexia nervosa (AN) are severe and disabling psychiatric disorders that share clinical features of distorted body image and overvaluation of appearance. An important shared clinical phenotype in BDD and AN is perceptual distortion of appearance, which may contribute to distorted body image. There is early evidence of common phenotypes of disturbances in visual perception and visuospatial processing in BDD and AN, as

evidenced clinically and from neuropsychological testing. Functional magnetic resonance imaging (fMRI) studies in BDD demonstrated abnormal activation in left hemisphere regions responsible for high-detail processing when viewing others' faces, and relative hypoactivation for visual cortical regions for configural/holistic information when viewing own-faces. Neuroimaging studies in AN examining visual processing of others' bodies have found abnormalities of frontal, parietal, and limbic regions, and the fusiform gyrus. Despite their significant morbidity and mortality, no study has directly compared and contrasted the neurobiology of these disorders in order to understand the underlying shared and unique phenotypes. The objective of this fMRI study was to investigate the neural correlates visual processing of others' bodies using different types of visual stimuli that convey high, low, or normal level of detail.

Methods: Subjects: Participants included right-handed males and females with DSM-IV BDD (N=12), AN (N=5), and healthy controls (N=9), matched by age and gender. The subjects with AN met all DSM-IV criteria (aside from amenorrhea), except they were required to have a BMI of ≥ 18.5 , in order to avoid confounds from the starvation state. Stimuli and Task: Participants engaged in matching tasks of photographs of bodies. There were three subsets of photographs: unaltered, containing only high spatial frequency (high detail) information, or containing only low spatial frequency (low detail) information. We used a blocked design with each stimulus type contrasted to the control task of matching shapes to investigate brain regions activated by the stimuli. Eye-tracking data were collected to ensure subjects were viewing the stimuli and to investigate visual fixations as a covariate. fMRI: We used a 3-Tesla Trio (Siemens) MRI scanner to evaluate BOLD contrast, using T2*-weighted echo planar imaging. We also obtained a matched bandwidth T2 scan and a high resolution T1-weighted MP-RAGE scan for each subject to provide detailed brain anatomy during structural image acquisition, for a two-stage registration. Data Analysis: FSL provided the tools for preprocessing, registration, and analysis. We modeled the responses to stimuli using the general linear model. A one-way ANOVA was performed to test differences in means among the three groups, followed by pairwise comparisons. We thresholded Z statistic images using clusters determined by $Z > 1.7$ and a corrected cluster significance threshold of $P = 0.05$.

Results: For all image types, the BDD group demonstrated greater activation than controls in right temporal-occipital or occipital fusiform cortex. The AN group demonstrated lesser activation than controls in the left temporal-occipital fusiform cortex for the unaltered images and greater activation than controls in left lateral occipital cortex and left temporal-occipital fusiform cortex for the low detail images. The BDD group demonstrated greater activation than the AN group in the left temporal-occipital fusiform cortex for the unaltered images, greater activation in the left temporal-occipital fusiform cortex and left lingual gyrus for the low detail images, and greater activation in bilateral occipital fusiform cortex for the high detail images.

Discussion: BDD and AN subjects demonstrated abnormalities in ventral visual pathways when processing bodies, including body selective regions of the fusiform cortex. Specifically the BDD group demonstrated abnormal hyperactivity for all image types while the AN group demonstrated abnormal hypoactivity for unaltered photos and hyperactivity for low detail images. The BDD group demonstrated greater activation relative to the AN group in ventral visual pathways. Limitations of this study include small sample size, especially for the AN group. Nevertheless, these results suggest early evidence that these related disorders of body image may be associated with abnormalities in secondary visual processing systems.

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167. Cortical Structural Abnormalities in Deficit Versus Nondeficit Schizophrenia

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Background: People with the deficit form of schizophrenia, characterized by primary, enduring negative or deficit symptoms, represent a distinct subgroup within the syndrome of schizophrenia. There is limited information on the neuroanatomical substrate of deficit symptoms. Although behavioral and functional neuroimaging studies have implicated the dorsolateral prefrontal thalamocortical circuit in deficit schizophrenia, results from structural imaging studies have been mixed. We examined the frontal, parietal, and temporal areas of the heteromodal association cortex (HASC) in deficit and nondeficit schizophrenia and a healthy comparison group. We hypothesized that the deficit group would be characterized by distinct volume abnormalities in the prefrontal and inferior parietal cortices.

Methods: The study included 20 deficit and 36 nondeficit outpatients with schizophrenia and 28 individuals in a healthy comparison group. A spoiled gradient recall acquisition in steady-state three-dimensional magnetic resonance imaging sequence was used for the morphometric assessment of the HASC and select subcortical structures. Cortical images were color-coded by region of interest and measured using a 3D Cavalieri grid that represented volume of voxels. Subcortical volumes (thalamus, caudate, hippocampus, amygdala) were calculated by summing the area measurements across all appropriate images and multiplying by slice thickness.

Results: The superior frontal gyrus gray matter and superior and middle temporal gyri gray matter were significantly smaller (all $p < 0.05$) in the deficit group [$19.47\text{cc} \pm 1.01$; $13.28\text{cc} \pm 0.64$; $7.42\text{cc} \pm 0.45$] versus both the nondeficit [$22.04\text{cc} \pm 0.80$; $14.91\text{cc} \pm 0.51$; $9.33\text{cc} \pm 0.36$] and control groups [$22.72\text{cc} \pm 0.83$; $15.12\text{cc} \pm 0.53$; $9.28\text{cc} \pm 0.38$]. There were no significant nondeficit/healthy comparison group volume differences in these regions. There were no group differences in any inferior parietal cortical volume. The groups did not differ on total cranial, total brain, total ventricular, or total CSF volumes. There were no deficit/nondeficit group differences in examined subcortical structures. Deficit and nondeficit schizophrenia groups did not differ on ratings of positive symptoms or duration of illness.

Discussion: The current results suggest that people with the deficit form of schizophrenia are characterized by selective reductions in the prefrontal and temporal cortex. The lack of deficit/nondeficit morphometric differences in subcortical structures suggests that these regions are not implicated in the neuroanatomy of deficit symptoms.

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168. Frontal-Amygdala Functional Connectivity Increases from Mania to Euthymia in Adults with Bipolar Disorder: A Longitudinal Functional MRI Study

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Background: Abnormal functioning within the frontal cortex and amygdala has been reported in functional magnetic resonance imaging

(fMRI) studies of adults with bipolar disorder (BD). However, fMRI activation does not fully address the functional connections between these brain regions. Herein we assessed the strength of the relationship in time between the blood oxygen level-dependent (BOLD) response in bilateral amygdala and all other brain regions on a continuous performance task with emotional and neutral distracters (CPT-END) using a voxel-wise functional connectivity analysis. We compared the same participants with BD across manic and euthymic (relatively symptom-free) mood states in longitudinal fashion and predicted greater amygdala connectivity, most likely with frontal lobe regions, during euthymia relative to mania.

Methods: Fifteen participants with BD were recruited from the University of Cincinnati Academic Health Center during an acute manic episode. All participants were evaluated using the Structured Clinical Interview for DSM-IV and a variety of symptom severity rating scales. Participants then received an fMRI scan during the index manic episode and a second follow-up scan up to one year later during a euthymic period. All participants were scanned at the University of Cincinnati College Of Medicine's Center for Imaging Research using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system. During both scans participants performed a CPT-END task. This task is a visual oddball paradigm with the addition of emotional and neutral pictures taken from the International Affective Picture System (IAPS). A functional connectivity analysis was performed using the left and right amygdala as seed regions. For each participant, a mean time series for the left and right amygdala was calculated by averaging the time series for all voxels within each region of interest. Analyses were then performed between the mean amygdala time series and the time series for each brain voxel, resulting in activation maps for each participant. These maps were then combined across participants within groups using a voxel-wise one-sample t-test to produce whole brain composite maps. Contrast maps to assess between-group differences were then created using voxel-wise dependent-sample (euthymic vs. manic) t-tests. Exploratory whole-brain analyses between participants while in manic and euthymic mood states were considered significant for $p < 0.05$ (corrected), cluster size > 36 contiguous voxels.

Results: Study participants were 30 years old with 14 years of education on average and all were receiving psychotropic medications. There were no significant performance differences on the CPT-END across mood state. Whole-brain analyses revealed greater functional connectivity in euthymia relative to mania between left amygdala and: (1) left anterior cingulate (cluster = 49 voxels, center of mass: $x = 20\text{ mm}$, $y = -41\text{ mm}$, $z = -6\text{ mm}$, $t = -3.21$), (2) right medial frontal gyrus (cluster = 43 voxels, center of mass: $x = -1\text{ mm}$, $y = -52\text{ mm}$, $z = 1\text{ mm}$, $t = -4.08$). Additionally, there was greater functional connectivity in mania relative to euthymia between left amygdala and: (1) left inferior parietal lobe (cluster = 142 voxels, center of mass: $x = 52\text{ mm}$, $y = 44\text{ mm}$, $z = 39\text{ mm}$, $t = 4.10$), (2) the border of the right middle cingulate gyrus (cluster = 49 voxels, center of mass: $x = -18\text{ mm}$, $y = 27\text{ mm}$, $z = 25\text{ mm}$, $t = 3.50$).

Discussion: These results provide evidence for changes in frontal-amygdala functional connectivity across mood state during emotional processing in BD. In that these connectivity difference occurred in the same individuals across manic and euthymic episodes, they are likely to represent valid neurofunctional markers of mood state. Greater functional connectivity between amygdala and regions of the frontal lobe during euthymia may represent a return of cortical regulation during periods of mood stability. Greater functional connectivity between amygdala and posterior cortical regions during mania may represent compensation for diminished processing in the anterior-limbic brain network necessary for emotional and cognitive homeostasis.

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169. Idle Minds Are the Devil's Playground: Auditory Hallucinations in Schizophrenia

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Background: The old adage, "an idle mind is the devil's playground" is being challenged by neuroscientists who are reporting that not only is the "idle mind" busy, but it may be busy consolidating new information and preparing for upcoming events. This undirected mind wandering can include unbidden memories of old conversations and imagined ones. In normal people, these auditory percepts would be tagged as coming from "self", but in most patients with schizophrenia, they might be perceived as "voices", often coming from external sources. Many of the same brain structures that support normal inner speech also support pathological voices; spontaneous activity in left primary auditory cortex is seen during resting scans in healthy people(1). We found that auditory cortical areas of patients who hallucinate were "tuned in" to hear internal auditory channels, at the cost of processing external sounds; schizophrenia patients who hallucinated had less activation to probe tones in left primary auditory cortex than non-hallucinators(2). We suggested auditory cortex of patients, who tended to hallucinate, was "busy" tuning into voices. We now ask if brain structures typically engaged while "idling" in the default mode network (DMN) are more likely to include auditory cortex in people who experience auditory verbal hallucinations.

Methods: We collected fMRI data from 26 patients within 5 years of their first psychotic episode and 31 controls, during a 6-minute Rest period. Using the CONN toolbox of Whitfield-Gabrieli (<http://web.mit.edu/swg/software.htm>), we correlated the time-series in every voxel in the brain with the time series from the posterior cingulate/precuneus (PCC), a prominent DMN region(3). We entered auditory hallucination severity ratings from the Scale for the Assessment of Positive Symptoms as a covariate, and to control of general severity of illness, we also entered severity of negative symptoms from the Scale for Assessment of Negative Symptoms(4,5).

Results: As expected, in both patients and controls, activity in the PCC was temporally correlated with activity in other prominent regions of the DMN, including medial prefrontal cortex and inferior parietal lobe bilaterally(6). As predicted, patients had more connectivity between PCC and auditory cortex than the controls. Finally, the recruitment of auditory cortex into the DMN is likely carried on the heads of the patients who hallucinate, as we found that patients who experience more severe hallucinations have greater connectivity between PCC and auditory cortex ($p = .001$) even when controlling for negative symptom severity.

Discussion: The posterior cingulate/precuneus region (PCC) of the brain may serve the adaptive function of continuously sampling external and internal environments(3). Our data suggest that PCC may

recruit auditory cortical structures into this "search light" function in people who experience auditory verbal hallucinations. This connection may reflect a hypersensitivity to unbidden auditory verbal experiences. **References:** 1. Hunter MD, Eickhoff SB, Miller TW, Farrow TF, Wilkinson ID, Woodruff PW. Neural activity in speech-sensitive auditory cortex during silence. *Proc Natl Acad Sci U S A*. Jan 3 2006;103(1):189-194. 2. Ford JM, Roach BJ, Jorgensen KW, et al. Tuning in to the voices: a multisite fMRI study of auditory hallucinations. *Schizophr Bull*. Jan 2009;35(1):58-66. 3. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A*. Jan 16 2001;98(2):676-682. 4. Andreasen NC. The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA: University of Iowa; 1983. 5. Andreasen NC. Scale for the Assessment of Positive Symptoms. Iowa City, IA: University of Iowa; 1984. 6. Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJ. Default-mode brain dysfunction in mental disorders: a systematic review. *Neurosci Biobehav Rev*. Mar 2009;33(3):279-296. **Disclosure:** **J. Ford:** *Part 1*; Astra-Zeneca. *Part 2*; Astra-Zeneca. *Part 3*; Astra-Zeneca. *Part 4*; Astra-Zeneca. **B. Roach:** *Part 1*; Astra-Zeneca. *Part 4*; Astra-Zeneca. **H. Shanbhag:** None. **L. Rachel:** None. **V. Sophia:** None. **D. Mathalon:** *Part 1*; Astra-Zeneca. *Part 4*; Astra-Zeneca.

170. Longitudinal Brain Development from Birth to Age 2 Years

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Background: Brain development in the first years of life is critical for normal cognitive development as well as for risk of neurodevelopmental disorders such as schizophrenia and autism. Very little is known about structural brain development in this important period. Longitudinal studies of brain development in early childhood are technically challenging given the enormous growth of the brain, dramatic changes in tissue contrast, and ongoing myelination of white matter.

Methods: Longitudinal structural MRI scans were obtained on 73 typically developing children shortly after birth, and at ages 1 and two years on a 3T scanner head only scanner (Siemens). Children were scanned unsedated during a natural sleep. We applied a novel 4D longitudinal warping approach for segmentation and 90 region parcellation that involved 1) the intensity-based segmentation of two-year-old brain images as well as the creation of a subject-specific tissue probabilistic atlas, 2) the probabilistic atlas based tissue segmentation on the images acquired at two weeks and one year of age.

Results: There were marked regional differences in gray matter development in the first two years of life. Overall, regions of occipital, parietal and temporal cortex had the greatest growth in volume over the first two years of life, on the order of 160% and greater. Frontal and prefrontal cortex grew less rapidly, with an interesting pattern of slow growth in the first year of life and more rapid growth in the second. Subcortical structures such as the hippocampus, amygdala, thalamus and caudate grew least in terms of overall volume expansion - about 130% in the first two years of life. While there were gender differences in overall ROI volumes, with males having larger volumes than females, there were very few regions with gender differences in relative growth patterns.

Discussion: There is enormous growth of cortical and subcortical gray matter in the first two years of life with clear regional differences in cortical gray matter development. These regional differences in growth are consistent with known regional differences in synapse development in the first years of life, as well as with differential developmental trajectories of early sensory-motor development compared to later development of executive function. The ability to discern regional differences in gray matter development in early childhood provides a basis for understanding potential abnormalities of early brain development in disorders such as schizophrenia and autism.

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171. Gene by Disease Interaction on Orbitofrontal Gray Matter Volume in Cocaine Addiction: Impact of life-time use

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Background: Chronic cocaine use has been associated with structural deficits in brain regions having dopamine receptive neurons. However, the concomitant use of other drugs and common genetic variability in monoamine regulation present additional structural variability. We therefore examined variations in gray matter volume (GMV) as a function of lifetime drug use and the monoamine oxidase A (MAOA) genotype in individuals with cocaine use disorders (CUD) and healthy controls.

Methods: We compared 40 men with CUD with 42 male controls scanned with magnetic resonance imaging to assess GMV with voxel-based-morphometry. All individuals were genotyped for the functional polymorphism in the promoter region of the MAOA gene with "high" and "low" alleles. The impact of cocaine addiction on GMV was tested by 1) comparing CUD with controls, 2) testing diagnosis-by-MAOA interactions, and 3) correlating GMV with lifetime cocaine and alcohol use and testing their contribution to GMV beyond other factors. These analyses were conducted in SPM5 with a threshold of $p < .05$, False Discovery Rate corrected and with extracted volumes from the main effect results.

Results: 1) Individuals with CUD had reductions in GMV in the orbitofrontal (OFC), dorsolateral prefrontal (DLPFC) and temporal cortex, and hippocampus, compared to controls ($F_{1,72}$ ranging from 5.3-27.5, $p = .05-.0001$). 2) The OFC reductions were uniquely driven by CUD with the low MAOA genotype ($F_{1,68} > 5.2$, $p < .005$) and by lifetime cocaine use ($r = -.44$, $p < .005$). 3) GMV in the DLPFC was driven by lifetime cocaine and alcohol use; in the hippocampus, GMV was driven by lifetime alcohol use ($r = -.46$ and $.41$, respectively, $p < .005$). These findings were confirmed by multiple regression analysis where the contribution to GMV of demographic, MAOA genotype, and drug use variables were assessed. The resulting R^2 ranged from .36-.55, indicating that a combination of factors can explain nearly half of the variability in GMV in CUD and that lifetime cocaine and alcohol use are chief contributors to the GMV decrements.

Discussion: The regions found to have reduced GMV in CUD in the current study are associated with drug craving and drug seeking behaviors. Since the OFC and hippocampus, in concert with DLPFC regions, have an important executive role in inhibiting previously acquired drug reward mechanisms, these GMV decrements may perpetuate the Impaired Response Inhibition Salience Attribution (I-RISA) syndrome in drug addiction. We report for the first time, the enhanced sensitivity of CUD low MAOA carriers (CUD-L) to GMV loss, specifically in the OFC. This gene-by-disease interaction indicates that CUD-L show exacerbated effects of cocaine in the OFC, a region central to reward attribution and to self-control (i.e., I-RISA). The mechanisms by which the MAOA low allele interacts with cocaine use to selectively diminish OFC in the current study remains unknown. The modulating effect of the MAOA genotype on structural variability may have started during early brain development, continuing its impact at adolescence possibly expediting onset of disease progression. Interestingly, CUD-L in this study had a slightly younger age of onset for cocaine use. It is possible that these particular individuals who later developed CUD had reduced GMV in the OFC before disease onset, since developmental factors such as maternal smoking are associated

with increased likelihood of drug experimentation and decreased thickness of the OFC in adolescence. In this context, it is noteworthy that MAOA-L genotype is associated with risk for alcoholism and antisocial alcoholism. Lifetime alcohol use was the major contributor of GMV deficit in the DLPFC, temporal cortex and hippocampus of CUD, contributing unique variability to GMV above and beyond the MAOA polymorphism and any of the other factors tested and more so than cocaine use. Animal models of binge drinking support a direct link between high levels of alcohol consumption and neurotoxicity in the hippocampus during adolescence. Similarly, reduced hippocampus volume was found among adolescents with alcohol use disorders. Gray matter loss in the hippocampus may lead to enhanced drug seeking and more self-administration, further facilitating a vicious cycle of cocaine use.

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172. Developmental Alterations Of Cortical-subcortical Connectivity In Obsessive Compulsive Disorder

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Background: Atypical development of cortical-subcortical connections has been posited to contribute to obsessive compulsive disorder (OCD) on the basis of structural abnormalities in the striatum, thalamus, and prefrontal cortex in pediatric patients. However, age-related changes in resting state connectivity of subcortical to cortical regions remains to be studied in OCD.

Methods: Resting state functional connectivity data were collected in 60 patients with OCD and 62 healthy subjects, ranging in age from 8 to 40 years. Subjects were categorized as children (8 - 12 years), teens (13 - 17 years), younger adults (18 - 25 years) or older adults (26 - 40 years). Whole-brain voxelwise analyses in SPM5 tested for group effects on the connectivity of anatomically defined seeds in the ventral caudate (vCau) and medial dorsal thalamus (mdThal). Z-scores from areas of group differences were extracted to test for the effects of age and group x age interactions.

Results: Compared to healthy controls, patients exhibited increased connectivity of left vCau to mOFC (9, 69, 3; $k = 9$; $Z = 3.53$) and decreased connectivity of bilateral mdThal to the rostral ACG (left mdThal: -3, 45, 3, $Z = 3.71$; rmdThal: -3, 45, 6, $Z = 4.20$). Decreased left mdThal -rostral ACG connectivity [$F(1, 122) F = 2.3$, $p = 0.08$] tended to be more pronounced in younger patients, while increased left vCau-mOFC connectivity tended to be more pronounced in older patients [$F(1, 122) F = 2.3$, $p = 0.08$]. Planned contrasts revealed group differences for the left mdThal -rostral ACG in children and teens ($t = 2.9$, $p = 0.01$; $t = 2.2$, $p = 0.04$), while differences in left vCau-mOFC connectivity occurred in older adults ($t = -4.0$, $p < 0.01$).

Discussion: Reduced left mdThal-rostral ACG connectivity could represent an early marker for OCD, whereas increased left vCau-mOFC connectivity may develop over the course of OCD as patients age.

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173. Instantaneous Stress Perception (ISP) During the Trier Social Stress Test (TSST)

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Background: The TSST is the most potent social evaluative laboratory stress task for humans and has been used in clinical trials with pharmacological compounds over the last 10 years. The stress task

reliably provokes a response of the hypothalamus-pituitary-adrenal (HPA) axis response and the sympathetic nervous system (SNS) as well as an increase in perceived stress and anxiety. For determination of pharmacodynamic action of anxiolytic effects, pre-TSST measures (baseline) are usually compared with post-TSST measures. This excludes information of perceived emotions during the stressful event itself. Here we report information on perceived stress assessed during the TSST and relate our results to the physiological stress response.

Methods: 260 healthy males (age 16-60) were exposed to the TSST (Kirschbaum, Pirke, & Hellhammer, 1993). Subjects were asked to rate their stress perception on a visual analogue scale (VAS) shortly before entering the TSST, between both TSST tasks (ISP) and after TSST (post-TSST) completion. In addition, saliva cortisol samples were collected and heart rates (HR) were assessed. Effects of VAS values of each single time point (ISP and post-TSST) and VAS increase measures (ISP_{incr} and post-TSST_{incr}) were calculated. To explore the most sensitive measures, ISP values, post-TSST values and increase measures were compared with respect to their correlation with physiological parameters (cortisol and HR). In addition, for cortisol the "Area under the curve with respect to ground" (AUC_G) was calculated and a decline measure from ISP to post-TSST was generated.

Results: Perceived stress ratings were significantly increased during and after the TSST as compared to baseline values. However, subjects showed significantly higher perceived stress levels at ISP compared to the post-TSST value. When comparing the two increase measures with each other, ISP_{incr} was significantly higher as compared to post-TSST_{incr}. Heart rate and cortisol levels increased in response to the TSST. The ISP showed significant positive associations with HR increase, HR maximum, cortisol increase and AUC_G. In addition, there was also a positive relationship between ISP_{incr} and cortisol increase as well as HR maximum and HR increase. None of these associations could be shown for the post-TSST value and the post-TSST_{incr}.

Discussion: Here we introduce the observation that the instantaneous stress perception (ISP) during the first and the second half during the TSST represents the peak of perceived stress. Even though the TSST elevates reliably post stress measures of perceived stress, the ISP is the far more sensitive and easily obtained measure of perceived stress in response to the TSST. The ISP was also associated with the physiological stress response. Thus, the ISP adds valuable information on perceived stress that may add further clarity in anxiolytic drug development and clinical trials.

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174. Adverse Effects of Induced Hot Flashes on Sleep, Mood, and Quality-of-Life: A Gonadotropin-Releasing Hormone Agonist Model

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Background: Sleep disturbance and depression are common symptoms of the menopause transition with important deleterious effects on quality-of-life. While these symptoms are thought to be a consequence of hot flashes (or vasomotor symptoms, VMS), the impact of VMS on sleep and mood disturbance is poorly understood. Although VMS are associated with perceived sleep disturbance in most studies, the effect of VMS on sleep is controversial when sleep is measured objectively. Similarly, results of studies addressing the association between VMS and mood disturbance in perimenopausal women are mixed. We used

a gonadotropin-releasing hormone agonist (GnRHa) that induces hypoestrogenism and mimics the menopause transition to investigate the effect of VMS on sleep and mood by comparing changes in sleep and mood between those who did and did not develop new-onset VMS. **Methods:** The GnRHa leuprolide 3.75-mg/day depot was administered during the mid-luteal phase of one menstrual cycle to 20 healthy premenopausal volunteers without VMS, sleep disturbance, or psychiatric illness. VMS were assessed with a daily diary and objectively with a skin-conductance monitor during a one-month follow-up period. Serum levels of estradiol were measured weekly. Prior to GnRHa administration and at the end of follow-up, sleep was assessed objectively (2 consecutive nights of actigraphic monitoring) and subjectively (Pittsburgh Sleep Quality Index [PSQI]), depression symptoms were rated (self-reported Beck Depression Inventory (BDI) and clinician-rated Montgomery-Åsberg Rating Scale [MADRS]), and quality-of-life was assessed (Menopause-Specific Quality-of-Life Questionnaire [MENQOL]). Data were analyzed using non-parametric Wilcoxon-rank-sum testing.

Results: Of 20 women receiving GnRHa (age 30.6 ± 8.9 years), VMS were measured objectively in 15 (75%) women and 14 (70%) reported new-onset VMS, beginning after 8.4 ± 2.0 days on leuprolide. There was no significant difference between groups in age, race/ethnicity or body-mass index. Serum estradiol was suppressed to 15, suggestive of clinically significant depression. Post-treatment scores on the MENQOL subscales were also worse in those who developed new-onset VMS (psychological, $p=0.04$; physical, $p=0.06$; sexual function, $p=0.04$).

Discussion: Use of a GnRH agonist to induce hypoestrogenism and simulate the gonadal steroid hormone changes of menopause demonstrates that the development of VMS results in rapid deterioration of sleep, as measured by actigraphy and perceived sleep quality, as well as increased depressive symptoms and reduced quality-of-life. While severe insomnia and depression were rare, a significant proportion of women with VMS had meaningful worsening of sleep and mood. This study provides evidence that VMS have downstream effects on sleep and mood and suggests that VMS may play an important role in the genesis of sleep problems and mood disturbance in midlife women, although the mechanisms through which these effects occur remain unknown.

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175. Effects on Intranasal Oxytocin on Self-Aggression in Major Depressive Disorder

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Background: Oxytocin regulation of social and emotional behavior may have implications for suicidal behavior and targeted therapies for mood disorder. However, testing of hypotheses regarding oxytocin function in suicidal behavior raises methodological challenges. An alternative scientific approach is to use a laboratory measure of self-aggression, the Self-Aggression Paradigm (SAP), which has previously been linked to suicidal behavior. The SAP is a competitive computer game in which the subject may choose to shock themselves (or not) at varying levels of intensity, ranging from none to the highest shock level that they are willing to submit themselves to. We hypothesized that in male and female adults with current or past major depressive disorder, intranasal oxytocin administration would be associated with decreased self-aggression as reflected in decreased average self shock and decreased number of times subjects administer the highest shock level. Given that childhood trauma has previously been associated with

altered oxytocin function and major depressive disorder, the Childhood Trauma Questionnaire was administered and treated as a covariate.

Methods: All procedures were approved by the Institutional Review Board (IRB) of The University of Chicago, and all subjects provided written informed consent. Subjects underwent semi-structured SCID interviews for diagnosis of Axis I conditions, and completed the Childhood Trauma Questionnaire for calculation of CTQ Total score. 23 subjects with a past history of major depression, and 10 subjects with current major depression underwent a between-subjects experiment in which intranasal oxytocin (20 IU) or matching placebo were administered, followed 45 minutes later by performance on the Self-Aggression Paradigm (SAP). Outcome variables were threshold for lowest level of shock (Lo-Threshold), highest level of shock (Hi-Threshold), average shock level over all trials (Average Shock), and number of times the Hi-Threshold shock level was chosen (# Hi-Shock). Data were analyzed by MANCOVA, with the between subjects factors of Drug (Oxytocin, Placebo), Gender (male, female), and Depression (current, past), with CTQ Total score as a covariate.

Results: MANCOVA revealed that current depression was associated with higher Average Shock ($F(1, 27) = 5.011, p = .034$) and # Hi-Shock ($F(1, 27) = 5.810, p = .023$). Oxytocin was associated with larger # Hi-Shock ($F(1, 27) = 9.987, p = .01$) and at a trend level of significance, with elevated Hi-Threshold ($F(1, 27) = 3.147, p = .09$). A significant interaction was found between Depression and Drug for # Hi-Shock ($F(1, 27) = 4.256, p < .01$) and for Average Shock ($F(1, 27) = 4.256, p = .05$). The covariate, CTQ Total, positively predicted # Hi-Shock ($F(1, 27) = 5.322, p = .03$). Probing of the interactions by Depression subgroups revealed that in currently depressed subjects, oxytocin was associated with elevated # Hi-Shock ($F(1, 6) = 5.899, p = .051$). No effect was seen in the past depression group.

Discussion: Contrary to our hypothesis, intranasal oxytocin was in fact associated with an increased tendency towards self-aggression. The effect was seen primarily in subjects meeting criteria for current major depressive episode. Current depressive episode itself was associated with increased tendency towards self-aggression, as was history of childhood trauma. It is not clear why oxytocin may have an effect on self-aggression in adults with current depression. Altered pain threshold may be one mechanism. Further work is needed, as there may important public health implications of oxytocin regulation of self-aggression.

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176. Cortisol Responsivity to Social Stress Varies Across the Menstrual Cycle

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Background: The role of sex steroid hormones in stress responsivity is interesting in light of sex differences in stress responsivity. Men show increased cortisol reactivity to a well-validated social stress test (i.e., Trier Social Stress Test; TSST) compared to women. A sex difference in cortisol reactivity is not evident when stress is physical (e.g., exercise) or hormonal (e.g., corticotropin releasing hormone), suggesting that social evaluative threat is a critical factor in producing a sex difference. In the present study, we evaluated the influence of sex steroid hormones on stress responsivity by examining stress responsivity differs during distinct phases of the menstrual cycle, one characterized by low levels of endogenous estrogen and progesterone and the other by high levels. We also explored the impact of stress on memory retrieval for emotional words.

Methods: Thirty-seven naturally-cycling females between the ages of 18 and 40 participated in the study. Nineteen women completed the task during the midluteal (high estrogen and progesterone) phase and 18 during the follicular phase (low estrogen and progesterone phase).

Phase was validated by measuring plasma hormone levels. The two groups were similar in age (mean = 27 years), education (mean = 16), estimated IQ (mean = 106), race (49% white, 33% African American), depressive symptoms (mean = 8.5 on the Center for Epidemiological Studies Depression Scale) and anxiety symptoms (mean = 4.8 on the Beck Anxiety Inventory). Stress outcomes and memory measures were obtained first during a control condition and then during a stress condition. Stress was induced with the TSST, a well-validated laboratory stressor requiring public speaking. The control condition involved completing questionnaires. Stress outcomes included self-reported anxiety and stress (State-Trait Anxiety Scale, Visual Analog Scale), cortisol levels, heart rate, and heart rate variability. The emotional memory task began on the day prior to the TSST when participants learned two lists of word pairs to 100% criterion. Each list included five emotionally neutral word pairs, five positive word pairs, and five negative word pairs. Recall of the words was tested after a 24-hour delay on the day of the TSST, with one list tested after the control and the other list after the stress condition. The emotional memory task was structured to ensure that encoding was 100% and that the procedure addressed the impact of stress on retrieval.

Results: As expected the TSST produced greater stress responses than the control condition. Self-reported anxiety and stress increased, heart rate increased, and heart rate variability decreased during stress compared to the control condition (p 's < .01). The effect of stress on cortisol reactivity varied with menstrual phase ($p < .01$ for the interaction between condition and phase). Specifically, the impact of the stressor on cortisol reactivity was greater during the follicular versus midluteal phase ($p < .01$). Eighty-seven percent of women tested in the follicular phase were cortisol responders (defined by > 1.5 nmol/L) compared to only 37% of women tested in the midluteal phase ($p < .01$). Conversely, women tested in the follicular phase tended to report less anxiety compared to women tested in the midluteal phase ($p = 0.089$). With respect to emotional memory, women recalled fewer words following the stressor compared with the control condition ($p = 0.07$). Cortisol levels correlated negatively with recall of neutral, positive and negative words in the follicular phase only ($p < .01$).

Discussion: The present findings indicate that cortisol responsivity to a laboratory stressor varies across the menstrual cycle. Cortisol responsivity is lower when levels of the ovarian hormones estrogen and progesterone are high compared to when levels are low. Furthermore, it is only when ovarian hormone levels are low that cortisol levels predict recall of emotional words. These findings suggest a protective effect of ovarian hormones against the negative impact of cortisol on memory. These findings contrast with previous findings from Germany demonstrating that women resemble men with regard to cortisol reactivity at a menstrual phase when ovarian hormone levels are high. We found that instead, women resemble men when ovarian hormone levels are low. The reasons for this discrepancy are unclear but may in part reflect demographic and ethnic differences between samples.

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177. Spatial-temporal Transcriptional Responses During LPS-induced Inflammatory Stress.

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Background: Imbalance in the bidirectional communication between the central nervous system (CNS) and the immune system might increase the susceptibility to develop neuropsychiatric disorders. Peripheral administration of lipopolysaccharide (LPS) or IL-1 β in rodents elicits neuroinflammation, which causes depressive-like behaviour. Their effects can be inhibited by central, but not peripheral, administration of the endogenous IL-1 receptor antagonist (IL-1RA),

suggesting that the CNS is a key target for IL-1 β . It is therefore plausible that common molecular pathways are activated during inflammation and psychiatric disorders. With the aim of integrating the central and peripheral interplay between the neural, immune and endocrine systems, we determined the spatial-temporal progression of a group of transcripts that were differentially expressed between caspase-1 knock-out (casp1 $^{-/-}$), animals that lack mature IL-1 β , and wild-type (WT) mice in a model of LPS-induced systemic inflammatory response syndrome (SIRS).

Methods: We used virus and antibody-free mice (eight-week old, wild type males C57BL/6). Mice were kept in a light- (12 h on/ 12 h off) and temperature-controlled environment, with food and water ad-libitum. Injections were consistently performed between 7:00-9:00 h to avoid confounding factors due to circadian and/or ultradian rhythms. Mice were given intraperitoneal (i.p.) injections of either saline or 30 mg/kg E. coli (serotype 0111:B4) LPS (Sigma, St. Louis, MO) and sacrificed at different times between 0 to 24 h. Hippocampus, spleen, and adrenals were removed, snap-frozen, and stored at -80 °C until processing. Total RNA was extracted from each organ and converted into cDNA. Gene expression was determined by performing real-time PCR. Statistical differences were determined by performing one-way ANOVA.

Results: Our results showed that COX2 and NOS2 expression displayed the most striking transcriptional differences between casp1 $^{-/-}$ and WT. In the hippocampus of WT mice, COX-2 mRNA displayed a maximum of 3-fold increase at 2 h after LPS injection, remained elevated until 6 h ($p < 0.001$ vs. time 0) and declined at 12 h. A similar transcriptional pattern, but of lower magnitude, was also observed in casp1 $^{-/-}$ mice. At 6 h there were significant differences in COX2 mRNA levels between WT and casp1 $^{-/-}$ ($p < 0.01$). In the spleen of WT mice, there was a biphasic activation, with an early 2-fold increase at 1 h ($p < 0.001$ vs. 0 h) and a second delayed increase of the same magnitude at 6 h ($p < 0.001$ vs. time 0). In casp1 $^{-/-}$ mice, COX2 mRNA levels showed an early activation that peaked between 0.5 - 1 h ($p < 0.001$ vs. time 0), with no further increases at later times. At 6 h, COX-2 mRNA level was approximately 80% lower in casp1 $^{-/-}$ mice ($p < 0.001$). In the adrenal gland, the pattern of COX2 expression was similar to that of the spleen. NOS2 expression displayed a later activation in both brain and peripheral organs. In the hippocampus of WT mice, we detected a large increase of NOS2 expression at 6 h when compared to baseline ($p < 0.001$ vs. time 0). The same pattern was observed in casp1 $^{-/-}$ mice, but casp1 $^{-/-}$ displayed a smaller increase ($p < 0.001$ vs. time 0) when compared to baseline. At 6 h casp1 $^{-/-}$ mice displayed an approximately 40% lower levels of NOS2 mRNA when compared to WT mice ($p < 0.01$). At 6 h in the spleen, NOS2 mRNA expression had a 30-fold increase in WT mice ($p < 0.001$ vs. time 0), whereas in casp1 $^{-/-}$ it had an increase of approximately 13-fold ($p < 0.01$ vs. time 0). At 6 h casp1 $^{-/-}$ mice had a NOS2 mRNA activation that was about 80% smaller than that of WT mice ($p < 0.001$ vs. WT). In the adrenal glands, the pattern of expression was similar to those described for hippocampus and spleen, approximately 20-fold increase was noted at 6 h in WT mice ($p < 0.001$ vs. time 0) and a nearly 14-fold increase at 4 h in casp1 $^{-/-}$ mice ($p < 0.01$ vs. time 0). At 6 h casp1 $^{-/-}$ mice had a NOS2 mRNA activation that was nearly 40% lower than that of the WT mice ($p < 0.001$ vs. WT). Thus, inhibition of NOS2 activity might contribute to decrease SIRS-induced lethality.

Discussion: Collectively, these results suggest transcriptional changes in the brain and peripheral organs of casp1 $^{-/-}$ mice that could explain the resiliency to the deleterious effects produced by LPS-induced SIRS, and could consequently explain, at least in part, the better survival outcome of casp1 $^{-/-}$ mice to lethal doses of LPS. This line of research could lead to the development of novel therapeutic strategies to modulate the activity of casp1 during psychiatric and/or neurodegenerative conditions that involve activation of IL-1 β .

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178. Impact of Altering Electrical Parameters of Vagus Nerve Stimulation on Serotonin Neuronal Activity

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Background: Vagus nerve stimulation (VNS) is a non-pharmacological treatment used in drug-resistant depression. Studies have previously shown that VNS initially increases the firing activity and pattern of norepinephrine (NE) neurons, and subsequently those of serotonin (5-HT) neurons, via the activation of postsynaptic α_1 -adrenoceptors located on the soma of dorsal raphe (DRN) 5-HT neurons (1,2). Since these two neuronal systems are widely believed to underlie the antidepressant response, various stimulation parameters have been examined on their capacity to enhance 5-HT neuronal firing. These experiments, conducted in our laboratory, have demonstrated that VNS parameters used clinically, corresponded so far to the optimal ones that best activate 5-HT neurons in the rat brain (3). These last results suggested also that the use of the firing activity of 5-HT neurons is a reliable target to determine the optimal parameters of VNS in patients. Thus, using this paradigm, we have investigated further alterations of the stimulation parameters in order to optimize VNS efficacy in increasing 5-HT neurons activity, which could help enhance VNS efficacy in the treatment of depression.

Methods: Rats were implanted with a VNS electrode on the left side and a stimulator subcutaneously in the back area. The stimulator was turned on for 14 days using standard (0.25 mA, 20 Hz, 500 μ sec, 30 sec ON-5 min OFF, 24 hours a day) or modified stimulation parameters. The new parameters tested were 30 sec stimulation periods every 10 to 30 minutes as well as a 12-hour stimulation period per day, applied either during the light or dark period of the rat diurnal cycle using the standard parameters. In a second series of experiments, we tested a different modality of stimulation consisting of double or quadruple pulses at 5 msec intervals (200 Hz) delivered every 2 seconds for 36 seconds every 4.5 minutes 24 hours a day. Rat DRN 5-HT neurons were then recorded under choral hydrate anesthesia.

Results: Both 12-hour stimulation periods for 14 days, as well as the 30-second stimulation periods every 10 or 15 minutes significantly increased the firing activity of DRN 5-HT neurons to the same extent as the standard parameters. The 30-second stimulation periods every 30 minutes were not sufficient to enhance 5-HT neuronal firing. Finally, the stimulations in the burst mode significantly increased 5-HT neurons firing rate to the same extent as the standard stimulation parameters.

Discussion: These results showed that others stimulation parameters that are as effective as the standard ones in increasing the firing activity of DRN 5-HT neurons. Even if, the firing of 5-HT neurons was not increased further, these results demonstrate that less stimulation is sufficient to achieve the same VNS efficacy on 5-HT neurons. These data are important for patients using VNS because these new parameters of stimulation could help minimize or even prevent side effects of the treatment and increase the battery life of the stimulator. Furthermore, it would be interesting to determine if and how sleep architecture is affected when stimulating the vagus nerve for only 12 hours during the sleep-wake cycle.

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2. Manta et al, J Psychiat Neurosci 34:272-80, 2009

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179. Developing Strategies for Therapeutic Neuromodulation: Simultaneous Transcranial Magnetic Stimulation and Functional Magnetic Resonance Imaging to Study the Mechanisms of Hemispatial Neglect Recovery.

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Background: The clinical neurosciences are undergoing a significant paradigm shift driven by basic and translational discoveries of the structure and function of neural networks. The study of brain connectivity and the mechanisms that change it has become crucial to understanding the biological basis of behavior and neuropsychiatric disorders. As this paradigm advances, it provides not only a new theoretical framework but also new clinical tools, such as therapeutic neuromodulation. Systems and Cognitive Neuroscience have studied attention and its neural circuitry to a great detail. Hemispatial neglect is a neuropsychiatric syndrome characterized by the failure of the attentional network, usually due to an insult to the right parietal cortex, leading to spatial attentional deficits towards the contralesional side. The aim of this study is to explore the natural compensatory mechanisms of the brain to adapt to a brain lesion, specifically a parietal lesion. If we understand these reparatory strategies, we may be able to identify fundamental mechanisms of adaptive plasticity, and possibly facilitate them using neuromodulatory techniques.

Methods: In the present study we used the simultaneous combination of TMS and fMRI to create a model of Hemispatial neglect and image the effects of TMS "virtual" lesions during a task. We studied 8 healthy subjects while they performed a spatial attention task in the scanner. This multimodal approach required an MRI-compatible TMS coil placed over the right parietal cortex of subjects laying in the scanner. The task involved 5 different conditions during which subjects covertly attended to the left or right, attended to the left or right while receiving TMS over the right parietal cortex, or received TMS at rest. TMS pulses were synchronized with the MRI scanner via a separate computer and a customized program to avoid interference of the TMS magnetic field during the image acquisition process. The cognitive task was set at psychometric ceiling conditions to allow subjects to perform adequately even during TMS inhibition.

Results: First, we describe the net effects of TMS, which reveal the activation of a network of bilateral regions with known connectivity to the parietal attentional centers, in addition to the auditory and somatosensory cortex activated by the noise and tapping sensation of TMS. Second, our analysis compared (A) the "pathological condition" (attending under the influence of TMS) to (B) the "healthy condition" (attending without TMS) + the net effects of TMS at rest. Since condition A results in the addition of the 2 components of condition B, this comparison should lead to no significant changes, unless condition A represents not only the added effects of spatial attention and TMS, but also the brain's compensatory strategy to maintain functional competence despite the "virtual" TMS lesion. Because TMS did not disrupt performance of the attentional task (set at ceiling conditions), the changes of activation reflect only adaptive shifts in functional connectivity unbiased by a change in behavioral performance. The results describe a selective bilateral pattern of fronto-parieto-occipital activatory and inhibitory changes suggesting an adaptive response within the attentional network. Last, we repeated the second analysis with a more conservative statistical threshold to see the areas of maximum significance, and describe a contralesional focus of parietal inhibition. This suggests a possible neuromodulatory therapeutic strategy, by artificially augmenting the natural adaptive inhibition of this center with, e.g. image-guided neuromodulation.

Discussion: Our results propose a model to study the plastic strategies used by the brain to adapt to a lesion and maintain functional competence. Our paradigm models the conditions of a recovered parietal lesion (typically inducing Neglect), but could be easily translated to other systems and deficits. Understanding the changes in connectivity needed in order to maintain (or recover) function after

a brain insult may offer a roadmap of necessary steps that could be therapeutically induced by neuromodulatory interventions. Similar approaches could be used to study not only structural but also functional lesions in Neuropsychiatry.

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180. Noradrenaline Release Explains Effect of Vagal Nerve Stimulation in Pig Brain *in vivo*: PET Study with Tracer [¹¹C]yohimbine, an Alpha-2 Adrenoceptor Antagonist

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Background: Vagal nerve stimulation (VNS) is approved for the treatment of epilepsy and depression but the mechanism is not definitively established. Neuromodulatory effects of noradrenaline include inhibition of cortical excitability by means of noradrenoceptor activation, indicating that noradrenaline release may explain the beneficial effects of the stimulation. Hypothesis: Stimulation of the brain via vagal nerve afferent fibers in the neck leads to increase release of noradrenaline (NA) in cerebral cortex.

Methods: Longitudinal studies were performed in Göttingen minipigs. The treatment schedule included implantation of the VN stimulator during surgical anesthesia, followed by 6-8 weeks recovery. A baseline PET session with the stimulator OFF yielded the volume of distribution of the alpha 2 inhibitor yohimbine labeled with carbon-11. With an interval of 30 minutes, onset of stimulation at 1 mA was followed by a second session PET scan with [¹¹C]yohimbine, the decline of the volume of distribution of which was used as a surrogate marker of NA release. To determine binding potentials, we calculated the partition coefficient by means of an inhibition plot of volumes of distribution with stimulation on versus volumes of distributions with stimulation off.

Results: The acute VNS led to significant declines of the baseline volumes of distribution in thalamus and temporal and frontal cortices. The volumes of distribution yielded binding potentials by introduction of the partition coefficient of 0.95 ml/cc that yielded the non-specific accumulation of unbound yohimbine in the tissues. Binding potentials before and during stimulation averaged 4.0 and 3.4, respectively, in cerebral cortex. The change of binding potential was consistent with an increased occupancy of endogenous noradrenaline of 14% (standard error 2%).

Discussion: The decline of yohimbine binding potential during acute vagus nerve stimulation is consistent with release of noradrenaline that displaces the bound tracer yohimbine. The change of this putative increase of noradrenaline binding represented an occupancy of 14%. Binding potentials depend both on receptor density and receptor occupancy by other ligands. In this case, it is unlikely that acute VNS would lead to an instant change of receptor density. Thus, we conclude that the beneficial effects of VNS may be explained by the release of noradrenaline that lowers the excitability of cortical neurons.

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181. Depressed Patients Accommodate To The Painfulness Of Daily Left Prefrontal rTMS: A Replication Study In A Sham-Controlled Setting

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Background: In a prior analysis of an open label phase of the OPT-TMS study, we found that depressed patients rapidly accommodate to the painfulness of the TMS treatment over three weeks of treatment.(1) We wondered whether this effect would also be seen in the double-blind initial phase of the study, and whether there would be differences

in pain accommodation in those receiving active TMS compared to those who received the active sham which was matched for sensation and initial painfulness.(2)

Methods: We analyzed visual analog pain ratings acquired immediately before and after every TMS treatment session for all patients in the recently completed OPT-TMS trial (n = 199).(3)

Results: Confirming our observation in the open phase, patients overall in the double-blind phase rapidly accommodated to the painfulness of the procedure. Procedural pain ratings decreased significantly within the first session of TMS (mean pain at beginning of session = 52.3(SE = 5.8) out of 100; pain at end of session = 44.3 (SE = 4.9); a 15% reduction, $p < .05$). At the end of week-one, average procedural pain ratings decreased from 47.8 (session 1) to 31.7 (a 33% reduction; $p < .05$). Further analyses to be presented at the poster will test whether there were differences in those who received active or sham TMS, and whether the pain accommodation predicts or correlates with clinical antidepressant improvement.

Discussion: The accommodation to the painfulness of the TMS procedure likely is a factor in the high completion and low dropout rates in daily left prefrontal TMS studies to date. If the accommodation occurs more in those receiving active TMS, it may suggest that TMS is affecting prefrontal-limbic regulatory circuits to both reduce the perceived painfulness and, over time, reverse depression symptoms.

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182. Characterizing Drug Effects on Brain Network Activation: A Novel Analysis of EEG Evoked Response Potentials

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Background: Novel electroencephalographic (EEG) based approaches, which reflect millisecond temporal resolution of neuronal electrical activity, may provide novel insights into the impact of CNS-acting drug treatments on brain activity and function. The Brain Network Activation (BNA) algorithm can automatically detect the timing, order of activation, and dynamic orchestration of neural response to approved and novel drug treatments in healthy and disease populations (Pinchuk, 2009).

Objectives: 1) To assess electrophysiological effects of scopolamine (SC) and ketamine (KT) in comparison to placebo (PB) in healthy volunteers using BNA analysis. 2) To assess electrophysiological effects of single dose methylphenidate in adults with ADHD using BNA analysis.

Methods: *Study 1* The study followed a randomized; double-blind; double-dummy; PB controlled crossover design. Fifteen normal

controls (12 males; mean age 37.6 ± 5.6 yrs), received single doses of PB, saline, 100 mg KT, and 0.4 mg SC. Subjects underwent a baseline EEG scan followed by a second scan at 1h post dose. EEG tasks included sensory gating, auditory oddball, and working memory paradigm.

Study 2 Twenty-four subjects, 12 outpatients with ADHD and 12 age-matched normal controls (mean age 38 yrs) were enrolled. ADHD subjects met DSM-IV TR criteria. Any medications with clinically significant CNS effects were discontinued prior to EEG; no concomitant psychotropic medications or medications with CNS effects were allowed. ADHD subjects underwent baseline EEG scan 1-2 weeks before the drug session, in which they received a single 36 mg oral dose of an extended duration methylphenidate (OROS MPH), followed by a second EEG scan 2 hours post-dose. EEG task involved an auditory Go-NoGo paradigm. BNA algorithm was used to analyze 64-electrode EEG data which retrieved brain networks residing across multiple areas, frequencies and timescales. Pre-processed EEG data was band pass filtered into overlapping physiological frequency bands, epoched and averaged to ERPs. For each band, data were reduced into a set of discrete points to denote local extrema. For each condition the algorithm searched for synchronous peak latencies across subjects. Next, peak-pair patterns were identified, such that inter-peak intervals were also synchronous across subjects. More complicated patterns of ≥ 3 peaks were also identified, until a state-unique multi-site spatio-temporal pattern or several patterns emerged. Most distinguishing patterns were then used as opposing poles. Subject's were assigned similarity indices that quantified degree to which individual BNA pattern matched that of each pole, for each condition.

Results: *Study 1* Drug effects on spatio-temporal measures of neural activity automatically detected by BNA were seen for most EEG tasks ($p < 0.05$). Differences between KT and PB were found in N100 network following first auditory click (sensory gating task), which was lateralized to left hemisphere for PB. Differences were also seen in the frontal delta frequency negativity (~ 500 msec) preceding 2nd click for PB probably reflecting anticipatory response which was lacking following KT administration. Main differences between SC and PB networks were seen on working memory task, including delayed activation of the visual cortex corresponding to the N170 face processing component, and late frontal activation (~ 250 msec) which was more central and widespread after SC. In both cases, drug administration significantly reduced PB BNA scores. *Study 2* Pre-treatment Extracted BNA pattern for control subjects revealed a distinct left centro- parieto-occipital network in the delta frequency band. In contrast, pre-treatment ADHD subjects revealed activation in the fronto-central and midline regions. *Post-Treatment (36 mg OROS MPH)* Post-treatment activation patterns were altered following drug administration revealing a shift to right-hemisphere posterior-frontal network activation in predominantly delta frequency band (Mean ADHD BNA score 57 pre-dose vs. 41 post-dose, $p = 0.025$). ADHD post-dose BNA patterns also showed increased similarity to control BNA pattern (Mean control BNA score 28 pre-dose vs. 34 post-dose, $p < 0.05$).

Discussion: BNA analysis revealed significant changes in spatio-temporal measures of neural activity following drug administration. Results are consistent with known effects of anticholinergic (Thiel, 2002), dissociative (Oranje, 2009), and stimulant (Makris, 2009) drugs. These preliminary findings confirm BNA as a sensitive method for investigating drug effects on neural activity and may have potential as a biomarker of drug effects.

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183. Neuropsychological Tests As Predictors Of Fear Conditioning And Extinction.

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Background: There is increasing recognition that people with anxiety disorders show altered fear learning and fear extinction. Furthermore, evidence-based treatments for anxiety disorders usually consist of some form of extinction-based intervention such as exposure therapy. Therefore, it might be useful to evaluate fear learning and extinction when making treatment decisions for these disorders. Fear learning and extinction can be assessed with experimental fear conditioning, where a visual cue is paired with a mild shock to elicit increases in the skin conductance response (SCR). Because it is not practical to assess fear conditioning on a large scale, we sought to determine if simple psychological tests routinely used in clinics could predict fear responses. In fact, certain neuropsychological tests share neuroanatomical substrates with fear learning and extinction.

Methods: Forty-seven healthy adults living in San Juan, Puerto Rico (31 female and 16 male aged 21-54) were administered a series of tests that included the NEO-PI personality inventory, State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), the Multi-Source Interference Task (MSIT, a counting interference task), the Wisconsin Card Sorting Test (WCST) and an Emotional Stroop Task (EST) which required subjects to identify the color of words that had either neutral or emotional significance. All tests were administered in Spanish and validated for Puerto Rico. Subjects were then trained in an established fear conditioning and extinction paradigm (Milad et al., 2005), which assessed the SCR to pictures of a colored light associated with shock (CS+, CS-), and to the room in which the light CS was presented (context). On day 1, subjects received habituation, conditioning and extinction trials. On day 2, subjects were tested for memory of extinction (recall), and contextual renewal. The extent to which tests predicted the physiological responses was assessed with individual regressions, and then with predictive models using a combination of tests (multiple linear regressions). Adjusted R² were used to assess the predictive power of the models outside of our own sample.

Results: While individual tests showed poor prediction of fear learning and extinction, a combination of tests yielded significant predictive models. We were able to predict the response to the CS+ during conditioning and renewal using combinations of the STAI, BAI, NEO-PI, MSIT, trauma history, and sex (Cond: R² = 0.288, $p < 0.01$; Recall: R² = 0.398, $p < 0.001$). We were also able to predict responses to the recall context with the MSIT and sex (R² = 0.261, $p = 0.001$) and to the renewal context with STAI, NEO-PI, and sex (R² = 0.510, $p < 0.001$). Thus, simple psychological tests were sufficient to predict as much as 50% of the variance of the fear responses in our sample.

Discussion: Our findings suggest that a 15 minute battery of computer tests could function as a cost-effective tool for guiding therapeutic interventions. We are now studying a clinical population to determine the extent to which these tests are also predictive of fear learning and extinction in an anxiety disorders group. Longitudinal studies could evaluate if these tests could also serve as a screening tool for identifying individuals at-risk for developing an anxiety disorder.

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184. Balanced-Placebo Design with Marijuana: Drug and Expectancy Effects on Sexual Risk Decisions

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Background: Despite associations between marijuana use and risk-taking behaviors including reckless driving and risky sexual behaviors,

controlled research on marijuana's acute effects on risk taking and impulsivity is limited, and the mechanism whereby marijuana may increase such behaviors has not been established. Both the pharmacologic effect of delta-9-tetrahydrocannabinol (THC) and the expectancy that marijuana impairs judgment or makes one disinhibited may lead to deficits in controlled cognitive processing and possible increases in sexual risk taking under the influence of marijuana. In contrast to research with alcohol and nicotine balanced-placebo design (BPD), there is little known about the effects of marijuana expectancy (being told that marijuana was consumed) on behavior.

Methods: In a 2 X 2 instructional set (told THC vs. told no THC) by drug administration (smoked marijuana with 2.8% THC vs. placebo) between-subjects BPD experiment, we examined the marijuana stimulus expectancy effect independent of pharmacologic effect on 1) subjective effects, 2) decision-making related to sexual risk taking behaviors, and 3) behavioral impulsivity measures. Individuals were first tested under a baseline/no smoking condition and again under experimental condition. Participants were 136 weekly marijuana smokers (mean age = 21; 35% female; 65% Non-Hispanic Caucasian).

Results: Both instructional set and drug manipulation significantly increased subjective ratings of cigarette potency; liking, satisfaction, and feeling happier after the smoking; food craving and physical reactions on the ARCI marijuana scale. Significant expectancy X drug interaction effect ($sr^2 = .05$, $p < .01$) was observed on risky sexual decision-making measures, with those given THC and told THC rating negative consequences of risky sex as more likely than those told placebo ($B = 1.26$, $SE = .57$, $sr^2 = .07$, $p < .05$). In the absence of THC, there was no significant association between instructional set condition and risky sex. Increased likelihood of risk from coercive sex was also seen among females told THC (vs. told no THC) ($sr^2 = .07$, $p < .05$). Significant pharmacologic and expectancy main effects were observed on the Stop Signal Task, where THC (vs. placebo) increased percent of missed responses on no-signal trials ($B = 5.18$, $SE = 2.44$, $sr^2 = .03$, $p < .05$) and marijuana expectancy (told THC) impaired reaction time on the task ($B = 55.12$, $SE = 26.27$, $sr^2 = .02$, $p < .05$).

Discussion: Awareness (or perception) of intoxication from the instructional set appears to have led to a compensatory response that may have counteracted the hypothesized disinhibiting effects of marijuana on risky sexual behaviors. Results illustrate the importance of considering expectancies in the context of drug administration studies and highlight the unique role of expectancy in marijuana-related sexual risk behaviors.

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185. Is Randomness Versus Determinism a Productive Question for the Neuromodulation? Specific Implications for Future Brain Stimulation Therapies in Depression

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Background: Nonlinear properties exist within the brain across a hierarchy of scales and within a variety of critical neural processes. Adaptive neuronal activity requires a dynamic range that provides sensitivity to a variety of different inputs and will thus self-organize as complex systems (Buzsáki 2006b; Kauffman 1993), at the critical point where dynamics are supple enough to respond to the external environment but stable enough to maintain homeostasis. More specifically, it has been hypothesized that improper self-organization could lead to an overall dysregulated system, performing in a too stiff or too chaotic range (Glass 2001). Brain stimulation therapies have shown promise in treating various psychiatric conditions, and in particular treatment-resistant depression. They offer a unique opportunities to efficiently and effectively optimize optimal mood regulation and sensitivity to stress.

Methods: We set out to characterize the non-linear properties of neuronal activity in 15 unmedicated depressed patients (age 39.4 ± 1.9 and 15 matched controls. Separately, we tested the effects of bilateral epidural cortical stimulation in 5 other depressed patients. Sixty-four channels surface EEG was acquired in a resting state. We specifically subtracted C16L-C16R and F14L-F14 R and studied the time series of differences. We focused on “symmetric sensor difference sequence” (ssds) thus emphasizing time, time scales, time structures i.e. phase space not real space representations.

Results: Unmedicated depressed and matched controlled significantly differed on Inventory for Depressive Symptoms (33.6 ± 1.9 , 4.9 ± 2.2 , $p = 0.001$). They also differed in all non-linear indices indicative. Epidural cortical stimulation for 60 seconds showed distinct phase shifts during and immediately after stimulation.

Discussion: Restricted or “stiff” neurodynamics appears characteristic of depressive states. More is needed to study the impact of brain stimulation and its effective long term treatment.

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186. Novel and Selective Dopamine D3 Receptor Ligands: Examination of the Carboxamide Linking Chain as a Point of Separability between D2 and D3 Receptor Binding

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Background: The dopamine D2-like receptor family, which is comprised of the D2, D3 and D4 receptor subtypes, has been a target for discovering medications for the treatment of neuropsychiatric and neurodegenerative disorders for decades. Studies have linked changes in the expression of dopamine D3 receptors (D3R) to progression of drug addiction and this has led to an interest in the development of D3R-selective compounds as potential medications to treat drug abuse (Heidbreder and Newman 2010 for review). Because of the high degree of amino acid sequence homology (78% in the transmembrane regions) between dopamine D2 receptors (D2R) and D3R, it has been challenging to obtain subtype selective agents. Structure-activity relationships (SAR) previously developed demonstrate the importance of the length and substitution on the linking chain between the aryl amide and the 4-phenylpiperazine in this class of D3R ligands. Previously, we have described a series of 3-OH-substituted-4-(4-(2,3-dichloro- or 2-methoxyphenyl)piperazine-1-yl)-butyl)-aryl carboxamides in which analogues with high D3R affinity ($K_i = 1$ nM) and (>400-fold) selectivity over D2R were discovered. In this series, the first enantioselective D3R antagonists were revealed in which enantioselectivity was more pronounced at D3R (15-fold) compared to D2R (<2-fold) (Newman et al., 2009).

Methods: In the present study, two new series of N-(3-fluoro-4-(4-(2,3-dichloro- or 2-methoxyphenyl)piperazine-1-yl)-butyl)-aryl carboxamides were designed, synthesized and evaluated for binding at hD3R and hD2R expressed in HEK 293 cells to 1) optimize for D3R affinity and selectivity and to 2) further characterize the role of the linking amide chain in this class of compounds.

Results: Binding studies revealed some of the most D3R selective compounds reported to date, (e.g. BAK 2-66 D3R $K_i = 6.1$ nM; D2R $K_i = 15,000$ nM.) To begin to define the molecular basis for this receptor subtype binding selectivity, we performed a series of radioligand binding studies using D3/D2 chimeric receptor proteins to identify what binding domains in the D3R as compared to the D2R were interacting with BAK 2-66 to achieve this magnitude of selectivity. These D2/D3 chimera receptor studies further identified the second extracellular (E2) loop as an important component that contributes to D3R versus D2R binding selectivity. Further, we discovered that while compounds lacking the carbonyl group in the amide linking chain bound with similar affinity to the amides at D2R,

this modification reduced binding affinities at D3R by >100-fold resulting in compounds with significantly reduced D3R selectivity.

Discussion: The 3-F substitution in the linking amide chain did not improve binding affinity for D3R as compared to the unsubstituted or 3-OH substituted analogues previously described, but dramatically reduced binding affinity at D2R. Further, reducing the amide carbonyl moiety to give tertiary and secondary amines decreased binding affinities at D3R, without effecting D2R binding. Hence, in addition to extending SAR at D3R, this study supports a pivotal role for 1) the E2 loop of the D3R and 2) the carbonyl group in the 4-phenylpiperazine class of compounds for binding, and further reveals a point of separability between D3R and D2R. These novel D3R compounds will provide important tools for both D3R structure-based discovery as well as *in vivo* tools for elucidating the role of D3R in addiction and the potential of developing D3 antagonists and partial agonists as medications to treat psychostimulant abuse.

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187. D-Cycloserine Augmentation of Cognitive-Behavioral Therapy in Pediatric Obsessive-Compulsive Disorder: A Preliminary Study

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Background: The N-methyl-D-aspartate (NMDA) receptor is critically involved in fear extinction, and the NMDA partial agonist D-cycloserine (DCS) has been shown to enhance extinction of learned fear in animal studies. Studies in human adults have shown efficacy of DCS augmentation of exposure therapy in acrophobia, social phobia, and panic disorder. Among adults with OCD, Wilhelm et al. (2008) showed medium between-group effect sizes in favor of DCS and Kushner et al. (2007) showed significantly more rapid reduction in obsession-related fear ratings. We examined the potential benefit and safety of DCS versus placebo augmentation of CBT in pediatric OCD patients, to our knowledge the first clinical study of DCS in youth with OCD.

Methods: Thirty youth with a principal diagnosis of OCD were recruited across two study sites between February 2007-December 2009. Participant characteristics are presented in Table 1. After obtaining written consent and assent, participants completed study measures, were administered a physical examination, had lab values assayed (CBC, metabolic panel, urine toxicology, and pregnancy test [for post-pubescent females]) and were randomized in a double-blinded fashion to CBT+DCS or CBT+Placebo. Assessments were conducted at pre-treatment, after session 6, and within one-week post-treatment. All participants received ten 60 min CBT sessions. As a dosage of 0.7 mg/kg was found to be effective in adult studies, two dosing levels were used based upon weight: children between 25-45 kg took 25 mg (0.56-1.0 mg/kg/day); and children between 46-90 kg took 50 mg (0.56-1.08 mg/kg/day) one hour before sessions 4-10. Measures included the CY-BOCS, ADIS-IV-P, CGI-Severity (CGI-S), Multi-dimensional Anxiety Scale for Children (MASC) and the Children's Depression Inventory-Short Form. Data were analyzed with separate 2 (site: Florida, MGH) by 2 (condition: CBT+DCS, CBT+Placebo) by 3 (time: pre-treatment, mid-treatment, post-treatment; Dependent variables: CGI-Severity, CY-BOCS Total Score) or 2 (site) by 2 (condition) by 2 (time: pre-treatment, post-treatment; Dependent variables: ADIS-CSR for OCD, MASC, CDI-Short Form) fixed-effects linear regression with time as the repeated measure. Cohen's d was used to examine the magnitude of treatment effect.

Results: There were no site differences across baseline demographics or clinical characteristics. Pre-treatment scale scores did not significantly differ as a function of group assignment (Table 2). For CGI-Severity ratings, we identified significant main effects for time ($F(2,27) = 86.8$; $p < .001$, $d = 3.5$) and group ($F(1,28) = 6.4$; $p = .02$,

$d = 0.97$). The group by time interaction was not statistically significant ($F(2,27) = 1.5$; $p = .22$); the effect size was moderate in favor of the CBT + DCS arm ($d = 0.47$) with a 57% versus 41% symptom reduction. Using the CY-BOCS, a significant time main effect ($F(2,27) = 118.4$; $p < .001$, $d = 4.1$) was identified. Although neither the main effect for group ($F(1,28) = 3.1$; $p = .09$) nor the group by time interaction ($F(2,27) = .69$; $p = .51$) met statistical significance, their effect sizes were moderate ($d = 0.66$) and small ($d = 0.31$), respectively. The average CY-BOCS reduction for the CBT + DCS arm was 72% versus a 58% symptom reduction for those randomized to CBT + Placebo. Using the ADIS-CSR, a main effect of time ($F(1,28) = 87.6$; $p < .001$, $d = 3.5$) was identified. There was no significant group or group by time interaction for MASC or CDI-Short Form scores (Table 2). No participant reported adverse effects related to DCS or placebo. CBC, LFTs, electrolytes and BUN, creatinine all were normal at enrollment and after treatment with DCS.

Discussion: Children randomized to DCS augmentation of CBT showed moderate treatment effects relative to a placebo control on several symptom severity indices. D-cycloserine was well-tolerated: no significant DCS-related adverse effects took place, and lab values did not change in treated youth. Despite several limitations, these preliminary data support study of 1) a fully powered trial applying DCS to CBT in pediatric OCD; 2) other pediatric anxiety disorders for which CBT is indicated; and 3) efficacy of DCS on alternative outcomes (e.g., participant attrition, treatment durability).

Disclosure: E. Storch: Part 1; Janssen Pharmaceuticals. Part 4; All Children's Hospital foundation, Obsessive Compulsive foundation, Tourette's Syndrome Association, Prader Willi Research Foundation, Rogers Memorial Hospital. T. Murphy: Part 1; Forest Pharmaceuticals, Janssen Pharmaceuticals. Part 4; Obsessive Compulsive Foundation, Tourette's Syndrome Association, All Children's Hospital Research Foundation, Endo. W. Goodman: None. G. Geffken: None. A. Lewin: International Obsessive Compulsive Foundation, Joseph Drown Foundation, Friends of the Semel Institute. A. Henin: Part 1; Shire Pharmaceuticals, Abbott Laboratories, Reed Medical Education/MGH Psychiatry Academy using independent medical education grants from Pharma. J. Micco: None. S. Sprich: Part 4; Michael Jenike endowed fund. S. Wilhelm: Part 1; Forest Laboratories, Reed Medical Education/MGH Psychiatry Academy using independent medical education grants from Pharma. Part 4; Obsessive Compulsive Foundation, Tourette's Syndrome Association. M. Bengtson: Part 1; Boehringer Ingelheim. D. Geller: Eli Lilly & Co. Speaker Bureau and Medical Advisory Board, Forest Pharmaceuticals, Glaxo Smith Kline, Pfizer, Otsuka, Boehringer Ingelheim, Alza, Bristol Myers Squibb, Novartis, Shire, Wyeth, Solvay, Lundbeck. Part 4; Obsessive Compulsive Foundation, Tourette's Syndrome Association, Wallace Foundation, McIngvale Family Foundation, Rogers Memorial Hospital.

188. Long-Term Efficacy & Effectiveness of Antipsychotic Medication for Schizophrenia, A Data-Driven Personalized, Clinical Approach Ira Glick*, John M Davis

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Background: There is currently unprecedented, international controversy about the relative efficacy of first- vs second-generation antipsychotics. The aims of this report are 1) evaluate the long-term (6 months or longer) relative efficacy and risk/benefits of first- and second-generation antipsychotics (FGAs and SGAs), and 2) suggest clinical guidelines on the optimal use of antipsychotics over the course of schizophrenia.

Methods: We performed a meta-analysis of all controlled studies of antipsychotics which were 6 months or longer. We also examined both naturalistic and first-episode studies to see if there was a pattern similar to results seen in our meta-analyses of short-term studies.

Results: The efficacy pattern of mid- to long-term studies was consistent with that of short-term studies. That is, clozapine,

risperidone, olanzapine, and amisulpride were superior to other antipsychotics. Side effect profiles varied by drug, but SGAs had less EPS and TD than FGAs. The SGAs differ in their propensity to increase weight with its metabolic consequences, to an elevation of prolactin, etc.

Discussion: Since schizophrenia lasts for life, most patients have had considerable previous experience with a variety of drugs, and also have their individual opinions and values about the various tradeoffs of efficacy and side effects among the drugs. We do not deal with picking the best antipsychotic for the average patient, but rather issues to be considered in shared decision making based on what has happened before. Over the long-term course of schizophrenia, some antipsychotics are more efficacious than others, and this greater efficacy is associated with better compliance and less relapse. For some patients, especially in the acute phase of the illness, side effects are thought to be equally or more important than efficacy. For chronic patients, efficacy is the defining issue for most patients and their families. The physician should be concerned about weight gain and its consequent metabolic issues and work with the patient on their management and prevention. Since patients will generally have the same side effects and the same efficacy on a given drug that they had in the past, and have their own idea about what drug they want or what side effect they can tolerate, we believe it is important that the clinician work with the patients and their families and caregivers to find the best tradeoff for them in the particular setting such as the availability of capacity to manage weight reduction, relapse prevention, and rehabilitation. We discuss the salient consideration to use in working with patients in shared decision making.

Hamburg MA, Collins FS. The path to personalized medicine. *The New England Journal of Medicine* 2010; 363(4):301-4.

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189. Riluzole, A Glutamate Antagonist, for Childhood Obsessive-Compulsive Disorder: Drug Serum Levels and Correlations with Adverse Effects and Efficacy

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Background: At the National Institute of Mental Health, as part of an ongoing trial testing the efficacy and safety of riluzole for OCD in children and adolescents, we elected to explore the potential relationships between serum levels of riluzole and the degree of symptomatic change and the side effects profile in our patients. We also investigated the relationship between the CYP1A2 genotype (a potential factor) and serum levels. CYP1A2 enzyme, a member of the cytochrome P450 family of enzymes, is the primary enzyme responsible for the metabolism of riluzole, with a lesser contribution from extrahepatic CYP1A1.

Methods: Out of 28 study finishers, riluzole blood serum levels were measured at weeks 16, 24, 36, and 52. (Riluzole levels were also measured during the double-blind phase of the trial, the first 12 weeks, but those levels remain blinded to date.) We analyzed the CYP1A2 alleles of 11 children on study drug to determine whether their genotype predicted their ability to metabolize riluzole, resulting in either higher or lower concentrations.

Results: We found no significant relationships between riluzole serum levels and improvements in global severity of illness, obsessions and compulsions, anxiety, or depression as measured by the Children's Global Impressions (CGI) scale, Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS), Multidimensional Anxiety Scale for Children (MASC), and Children's Depression Inventory (CDI), respectively. In the analysis from our trial's open-label phase, we also noted no correlations between mean serum levels and the side effects profile in each individual. No symptomatic adverse effects were noted, with the exception of two cases of pancreatitis. Laboratory

adverse effects (elevation of transaminases) also did not correlate with serum levels. Interindividual variation of riluzole serum levels was significantly larger than intraindividual variation, consistent with published literature on adult subjects. There was a wide range of riluzole concentrations (6.979 ng/mL–1158 ng/mL). Allelic differences in the CYP1A2 genotype did not correlate with the variability of riluzole serum concentrations in children taking the drug.

Discussion: This preliminary analysis was an attempt to account for apparent differences in symptomatic changes as well as laboratory adverse effects in subjects taking riluzole as a treatment for childhood OCD. Serum levels of unmetabolized drug were found to vary widely and were not correlated either with beneficial or adverse effects. Contrary to expectations, drug concentrations also appeared to distribute independently from CYP1A2 genotypes. The results are limited by the small sample size and lack of control over the timing of the blood collections, but suggest that these laboratory assays will be of limited utility in the management of children and adolescents treated with riluzole.

Disclosure: P. Grant: None. J. Song: None. E. Seligman: None. S. Swedo: None.

190. Nabilone Dose-Response in Marijuana Smokers: Comparison to Dronabinol

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Background: The synthetic cannabinoid receptor agonist, dronabinol (tetrahydrocannabinol; THC), decreases marijuana withdrawal symptoms, i.e., anxiety, marijuana craving, sleep disruptions, and anorexia, and decreases marijuana relapse in the laboratory when combined with the alpha-2 agonist, lofexidine (Haney et al., 2008). Nabilone is an FDA-approved synthetic analog of THC. Unlike dronabinol, nabilone produces urinary metabolites distinct from marijuana, allowing clinicians to distinguish ongoing marijuana use from nabilone compliance. Although this characteristic suggests that nabilone may have unique clinical utility, comparability of the dose-effect profile between nabilone and dronabinol has yet to be determined. Given the potential clinical use of cannabinoid agonists for the treatment of marijuana dependence, either alone or in combination with other medications, the objective of this study was to characterize nabilone's behavioral effects across a range of doses, and to compare these effects with those of dronabinol in current marijuana smokers.

Methods: Daily marijuana smokers (10M, 4F), averaging 5.4 + 3.8 marijuana cigarettes/day, completed this within-subject, randomized, double-blind, placebo-controlled study. Five additional participants (2M; 3F) did not complete the study: 1 did not like the medication effects and 4 did not follow study procedures. Over the course of 7 sessions, the subjective, cognitive and cardiovascular effects of nabilone (2, 4, 6, 8 mg), dronabinol (10, 20 mg) and placebo were assessed. Within each session, data were collected at baseline and at 30-60 minute intervals for 5 hours following capsule administration.

Results: Subjective Effects: Nabilone (4, 6, 8 mg) produced significant increases in ratings of Good Drug Effect and High, with peak effects occurring at an intermediate dose (6 mg), resulting in an inverted U-shaped function. Dronabinol (10, 20 mg) also significantly increased ratings of Good Drug Effect and High, with peak ratings for both doses comparable to those of the moderate nabilone dose (4 mg). Dronabinol had a more rapid onset of peak subjective effects (90-120 minutes post-capsule administration) compared to nabilone, which had peak effects 180-240 minutes post-capsule administration. Cognitive Effects: Nabilone (4, 6, 8 mg) dose-dependently worsened performance across a range of tasks designed to assess memory (Digit Recall), learning (Repeated Acquisition) and psychomotor function (Digit Symbol Substitution). Dronabinol (10, 20 mg) significantly worsened task performance on the Digit Recall and Repeated Acquisition Task,

comparable to the moderate nabilone dose (4 mg). Cardiovascular Effects: Nabilone (4, 6, 8 mg) dose-dependently increased heart rate and diastolic pressure, whereas dronabinol did not significantly alter either measure. Both nabilone (2, 4, 6, 8 mg) and dronabinol (10, 20 mg) significantly decreased systolic pressure.

Discussion: This study demonstrates that nabilone produces lawful, dose-dependent cannabinoid effects and has a longer time of peak onset than dronabinol. The medication was well-tolerated by marijuana smokers, supporting its further testing as a potential treatment for marijuana dependence. The lowest nabilone dose (2 mg) had few effects overall, whereas the 4 mg dose most closely mirrored dronabinol's (10, 20 mg) effects. Higher doses of nabilone (6, 8 mg) produced the most robust 'positive' subjective effects, disruption in cognitive performance, and cardiovascular changes. These dose- and time-course data can be used to guide dose selection in further clinical testing of marijuana smokers. This research was supported by US National Institute on Drug Abuse Grant DA09236.

Disclosure: M. Haney: None. G. Bedi: None. Z. Cooper: None.

191. Effect of Acute Post-Trauma Propranolol on Post-Traumatic Stress Disorder Outcome and Physiological Responses During Script-Driven Imagery

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Background: Animal and human research suggests that the development of post-traumatic stress disorder (PTSD) may involve the over-consolidation of memories of a traumatic experience. Previous studies have attempted to use pharmaceutical agents, especially the beta-adrenergic blocker propranolol, to reduce this over-consolidation. In this randomized, placebo-controlled study of the efficacy of propranolol in reducing the development of PTSD, we optimized dosages and conducted both psychophysiological and clinical assessments 1 and 3 months after the traumatic event.

Methods: Forty-one emergency department patients who had experienced a qualifying acute psychological trauma were randomized to receive up to 240 mg per day of propranolol or placebo for 19 days. At 4 and 12 weeks post-trauma, PTSD symptoms were assessed. One week later, participants engaged in script-driven imagery of their traumatic event while psychophysiological responses were measured.

Results: Although the participants who had received propranolol had lower physiological reactivity during script-driven imagery of their traumatic event at 5-weeks post-trauma than the placebo participants, the difference was not statistically significant. The rate of the PTSD outcome and the severity of PTSD symptoms were not different between the two groups. However, *post hoc* subgroup analyses showed that in the group of participants with high drug adherence, physiological reactivity was significantly lower during script-driven imagery.

Discussion: Although the physiological data provided partial support for a model of PTSD in which a traumatic conditioned response is reduced by propranolol, data from this study provide little evidence for clinical application.

Disclosure: E. Hoge: Part 4; Sepracor Inc.

192. Comparison of the Abuse Liability of Intranasal Buprenorphine versus Buprenorphine/naloxone in Buprenorphine-dependent Heroin Abusers

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Background: The abuse potential of intranasal (IN) buprenorphine/naloxone (bup/nx) in bup-dependent individuals is unclear given the unique pharmacology of bup. The present study was designed to assess

the abuse potential of IN bup/nx compared with bup in bup-maintained participants.

Methods: Heroin-dependent volunteers (N=5) participated in this randomized, placebo-controlled, double-blind study. Participants were maintained on 2, 8, and 24 mg sublingual bup for approximately two weeks at each dose. During a morning sample session, participants received \$20 and an IN dose of the test drug (bup, bup/nx, placebo, naloxone, or heroin). During an afternoon choice session, participants were given the opportunity to work for the test drug or money they sampled during the morning session. Each participant received all of the bup maintenance doses and all of the IN test doses.

Results: Ratings of drug liking and desire to take the drug again were lower for bup/nx than bup ($P < 0.05$). Participants also reported that they would pay less money for bup/nx compared to bup ($P = 0.016$) and heroin ($P = 0.0001$). Ratings of bad effects were higher for naloxone ($P = 0.0033$), 16/4 bup/nx ($P = 0.033$), and 8/16 bup/nx ($P = 0.0001$) compared to placebo. Self-administration of the bup/nx combination and of bup was lower than heroin (all $P < 0.014$) and did not differ from placebo.

Discussion: When administered IN, the bup/nx combination was not as well liked as bup. Self-administration of both bup/nx and bup was low. These preliminary results suggest a reduced abuse liability of bup/nx compared to bup and heroin in bup-maintained individuals.

Disclosure: J. Jones: Part 1; Reckitt-Benckiser, Schering-Plough. M. Sullivan: Reckitt-Benckiser, Schering-Plough. S. Vosburg: Reckitt-Benckiser, Schering-Plough. Z. Cooper: Reckitt-Benckiser, Schering-Plough. S. Comer: Reckitt-Benckiser, Schering-Plough.

193. Metabolomic Signatures for Response to Sertraline and Placebo
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Background: The treatment of Major Depressive Disorder (MDD) has changed dramatically over the years however, the detailed molecular mechanisms responsible for depression remain unknown. Response to current therapies varies considerably among individuals and the onset of antidepressant therapeutic action typically doesn't occur until after a few weeks of treatment making it hard to determine whether an antidepressant is going to work for a patient. The placebo effect adds complexity to clinical trials as up to 30% of MDD patients respond to placebo through mechanisms that are not yet understood. Metabolomics provides powerful tools to map biochemical pathways that are modified in disease and upon treatment of disease and could provide deeper insights into disease mechanisms, enable the sub-classification of disease, and yield valuable biomarkers for monitoring disease and the response to therapy. In this study we evaluated if metabolic profiles for MDD patients can be used to predict response to sertraline and to placebo.

Methods: MDD patients were randomly assigned to receive sertraline (up to 150 mg/d) (N=36) or placebo (N=40) in a double-blind four-week trial. Baseline plasma samples were profiled using the Liquid Chromatography Electrochemical Array; the output was digitized to create a "digital map" of the entire measurable response for a particular sample. Response was defined as $\geq 50\%$ reduction in baseline 17-item Hamilton Rating Scale for Depression total score. Models were built using training sets constructed with ca. 2/3 of the data from different parts of the HRSD17 response distribution, with the remaining 1/3 used for validation sets.

Results: Metabolic profiles separated responders and non-responders to sertraline or to placebo. These four groups were separated partially. Depending on the model, correct classification rate was 69-85% for sertraline and $> 80\%$ for placebo. Analyses of week 4 metabolic data yielded similar results. Baseline metabolic profiles suggested the presence of three subpopulations: 1) sertraline responders who would have also responded to placebo, 2) a group that responded to sertraline

but not placebo, and 3) a group that appeared to be unresponsive to both sertraline and placebo.

Discussion: The baseline metabolite of patients with MDD may be useful in predicting the acute response to four weeks of treatment with sertraline or placebo. Metabolomics may also help to define the biochemical basis for variations among patients in terms of response to sertraline.

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194. Antipsychotic Treatment and Tobacco Craving in People With Schizophrenia

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Background: Tobacco dependence is high in people with schizophrenia and about three times more prevalent than the general population. The risk of mortality in schizophrenia is doubled in smokers compared to nonsmokers. Many factors influence smoking in people with schizophrenia, however predictors of cigarette craving and related smoking behavior are not well established. Cigarette craving is known to be an important contributor to cigarette smoking, and clinical approaches that focus on regulation of craving are known to be effective in reducing rates of relapse. Nicotine mediates reinforcement from smoking via dopamine as dopamine transmission is related to levels of craving. Antipsychotics exert their effects by blocking dopamine, thereby potentially affecting craving and/or smoking behavior. Some data suggest the attenuation of craving by antipsychotics, while other data suggest increased smoking behavior with antipsychotic treatment.

Methods: We present data on 59 outpatients with a DSM-IV diagnosis of schizophrenia, between the ages of 18-65 years and considered smokers (at least 5 cigarettes per day and breath carbon monoxide (CO)(greater than or equal to 8 ppm). Subjects were given baseline assessments and one cigarette of their choice and then evaluated for smoking craving using the Tobacco Craving Questionnaire-Short Form (TCQ-SF) and breath CO 15 minutes post cigarette. The antipsychotic groups included N=19 clozapine, N=9 olanzapine, N=9 risperidone, N=10 first generation antipsychotics (FGA) and N=12 antipsychotic combination.

Results: Antipsychotic groups did not differ by age, race, sex, level of education, and marital status. There were no differences in cigarette smoking behavior such as number of cigarettes smoked per day (mean = 21.3 ± 11.6), CO levels (mean level = 28.3 ± 16.2 ppm) or scores on the Fagerstrom Test for Nicotine Dependency (FTND) (mean score = 5.4 ± 1.9). Fifteen minutes post cigarette mean scores on the total TCQ-SF were 48.9 ± 18.3 for clozapine, 48.8 ± 21.7 for olanzapine, 48.1 ± 17.8 for risperidone, 45.7 ± 21.3 for the antipsychotic combination and 36.5 ± 20.1 for the FGA group ($p = 0.028$ for FGA vs. clozapine). The subfactors for emotionality and expectancy were significantly lower with FGA versus clozapine ($p < 0.05$). Motivation to quit smoking for immediate reinforcement (measured by the Reasons for Quitting Scale) was rated significantly higher in the FGA

group compared to other antipsychotics ($F=2.57$, $df=4, 11.1$, $p=0.048$).

Discussion: In a cohort of heavy smoking schizophrenia subjects, those treated with FGAs rated their subjective craving to cigarettes to be lower than that of those treated with clozapine. Estimated craving by other SGAs was similar to that seen with clozapine. Specifically, these effects were noted in emotionality (i.e., subjective effects of improved cognition, irritability and control) and expectancy (i.e., subjective effects of enjoyment and pleasure). Thus, craving and smoking behavior with clozapine was not lower compared to other antipsychotics as previous research suggests. FGAs, antipsychotics having the strongest dopaminergic antagonism and lacking serotonin effects, were associated with the least amount of reported craving and strongest subjective rating for immediate reinforcement as a reason to discontinue cigarette smoking. This may be due in part to the secondary negative symptoms or dysphoria and lack of pleasure related to FGA treatment. It is important to consider antipsychotic effects on cigarette craving and smoking cessation motivation when considering smoking cessation strategies. More research is needed to better understand the disconnect between smoking behavior and dependency and decreased craving measurements demonstrated with this class of medications.

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195. Gender and Weight Moderate the Appetite and Food Consumption Responses to Methylphenidate in Healthy Adults

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Background: There is reliable evidence from animal studies, and some human research, that females show a greater behavioural response to stimulant drugs than males. It is believed that estrogen mediates these differences because it enhances the dopamine signal via inhibition of GABA neurons. One of the most common responses to stimulants is loss of appetite – as seen, for example, in those who use methylphenidate (MP) therapeutically for treatment of Attention Deficit/Hyperactivity Disorder (ADHD). This is a relevant issue because of the strong links between ADHD and obesity, and the recent evidence that stimulants are an effective treatment for intractable obesity in those with symptoms of ADHD. To date, we are unaware of any research assessing gender differences in the appetite response to MP.

Methods: In a sample ($n=132$) of adults, identified as obese or normal weight, we assessed appetite, cravings, and snack-food intake in response to a dose (0.5 mg/kg wt) of MP and placebo in a double-blind cross-over design. Data were analyzed using a 2 [Day: MP vs placebo] x 2 [Gender] x 2 [Weight Status] analysis of variance.

Results: There was a significant 3-way interaction for the three dependent variables (Snack Food Consumption: $p=0.014$; Appetite Ratings: $p=0.010$; Food Cravings: $p=0.008$) indicating a decrease in food-related responding in all groups from placebo to MP, *except* in obese males who showed no eating/appetite decrease to the MP challenge.

Discussion: These results could have a major impact for elucidating dopaminergic mechanisms in feeding and weight regulation. These mechanisms should provide targets for drug discovery for weight loss. Importantly, our work suggests that such dopaminergic anti-obesity medications would be utilized differently in males than in females.

Disclosure: C. Davis: None. R. Levitan: None. A. Kaplan: None. J. Carter: None. J. Kennedy: None.

196. Comparison of Efficacy and Safety Measures Among Pediatric (5) and Adult (23) Clinical Trials for Bipolar Mania Based on FDA SBA Reports Consisting of 5,201 Participants

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Background: There is evidence suggesting that success rates for adult trials are higher than for pediatric trials for major depression and generalized anxiety disorder. This raises concern regarding effectiveness of pharmacological therapy for children and adolescents. During the past decade several agents have been approved for the treatment of acute mania in the adult population. Little attention has been paid to trends in adult and pediatric response to drug and placebo arms from these trials. We therefore compared the efficacy measures, mortality rate, and incidence of severe adverse events reported in adult and pediatric Food and Drug Administration (FDA) Summary Basis of Approval reports between drug and placebo. We hypothesize that the efficacy and safety measures observed during the pediatric trials will be similar to that observed during the adult trials.

Methods: Over the past several years we acquired FDA SBA reports for 7 anti-manic agents all approved relatively recently for the treatment of acute mania in adults. The Pediatric Research Equity Act requires pediatric clinical pharmacology studies be conducted for agents approved for the adult population. The FDA Amendments Act requires expedited public availability of results from these studies as a matter of public health. We acquired Medical and Statistical Reviews detailing pediatric clinical trial data from 5 anti-manic agents. We calculated the mean Young Mania Rating Scale (YMRS) baseline score and change score for the drug and placebo arms from the pediatric and adult trials and the frequency of positive trials. We also calculated the number of adverse events that occurred in drug and placebo trial arms. We additionally calculated mortality rate per 100,000 person exposure years for both the pediatric and adult studies.

Results: The 5 pediatric studies involved 1,019 patients (716 drug/375 placebo). The 7 adult drug approval programs consisted of 23 clinical trials involving 4,110 patients (2,259 drug/1,851 placebo). The mean baseline YMRS score was 30.0 (identical) for both the adult and pediatric patients. The mean placebo arm change scores (-8.8 pediatric trials/-7.2 adult trials) and mean drug arm change scores (-14.1 pediatric/-12.3 adult) were similar across age groups. The success rate for the adult trials was 78% (18/23) and the success rate for the pediatric trials was 80% (4/5). There were no reported deaths during the pediatric trials. The FDA SBA reports reported 8 deaths (3 in drug group/5 in placebo group) during the 23 adult trials. This equated to an overall mortality rate of 3,653/100,000 per year. The incidence of severe adverse events amongst participants was slightly lower amongst the pediatric (3.9%) compared to adult (4.7%) patients. A similar frequency of severe adverse events was observed in the placebo (4.0% pediatric/5.1% adult) as compared to the drug (3.8% pediatric/4.5% adult) trial arms.

Discussion: These data suggest remarkable similarity in efficacy and safety data between adult and pediatric trials for bipolar mania. This profile is unlike the pattern seen in major depressive disorder and generalized anxiety disorder trials.

Disclosure: A. Khan: None. J. Faucett: None. W. Brown: None. R. Kolts: None.

197. Open-Label Uridine for Adolescent Bipolar Depression: a Magnetic Resonance Spectroscopy Neuroimaging Study

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Background: Pediatric bipolar disorder (BD) is a severe brain disease for which new treatments are urgently needed. Mood stabilizers and second-generation antipsychotics are first-line agents for pediatric

mania, but there is little evidence to inform treatment for depressed youth with bipolar disorder. Converging lines of evidence implicate changes in bioenergetic, i.e. mitochondrial function in the neurobiology of BD. One class of compounds with the potential to impact cerebral energy metabolism is the pyrimidines: these include cytidine, triacetyluridine and uridine. Pyrimidines that have been studied in adult bipolar depression include cytidine and triacetyluridine. In terms of safety, uridine is a constituent of human mother's milk, and is an ingredient in infant formulas. Magnetic resonance spectroscopy (MRS) is a neuroimaging technique for performing *in vivo* measurement of neuronal metabolites. MRS has been used to delineate the differences between untreated pediatric (BD) patients vs. treated patients and healthy controls. In clinical trials, MRS is used to determine the neurochemical changes associated with response to treatment. Uridine has been studied in adult bipolar depression, but data are lacking in youth. As an initial step, we treated seven adolescents with bipolar depression with open-label uridine. MRS brain scans were performed at baseline, and repeated after 6 weeks of treatment. Healthy control adolescents were recruited and scanned for comparison.

Methods: The study received IRB approval, and written parental consent and participant assent was obtained. The study was monitored by a Data Safety Board, and conducted under FDA Investigational New Drug Application #74,122. Inclusion criteria were: primary diagnosis of Bipolar Disorder I, II or NOS, a current depressive episode of > 2 weeks duration and a Children's Depression Rating Scale-Revised (CDRS-R) raw score > 40. Exclusion criteria included: active psychosis, high risk for suicidal behavior, Young Mania Rating Scale (YMRS) score > 10, positive pregnancy test, unstable medical condition or intellectual disability. Diagnoses were established with the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS-PL). A complete blood count, chemistry panel, lipid profile, thyroid stimulating hormone and urinalysis were obtained at baseline and repeated at the end of the study. Participants received fixed-dose uridine 500mg twice daily for 6 weeks. The following rating scales were administered at each visit: CDRS-R, YMRS and the Columbia-Suicide Severity Rating Scale (C-SSRS); adverse events were also recorded. A Siemens 3 Tesla MRI system and 31P/1H double-tuned volume head coil were used to acquire baseline and repeated measures 31-phosphorus (³¹P) MRS data from three participants and ten healthy controls. The data were analyzed using the jMRUI software package.

Results: Participants included five females and two males; all were Caucasian and not Hispanic. Five of the seven participants completed 6 weeks of treatment with uridine. Two participants withdrew from the study: in both cases, the decision was not based on efficacy or adverse events. The mean CDRS-R raw score at baseline was 65.6. The mean CDRS-R score for participants completing 6 weeks of treatment with uridine was 27.2. Last Observation Carried Forward (LOCF) analysis resulted in a mean final CDRS-R score of 30, or a mean reduction of 54%. No clinically significant laboratory abnormalities occurred. Adverse events were self-limited. No participant attempted suicide, engaged in self-harm, required hospitalization or experienced a manic switch. At baseline, no ³¹P-MRS neurochemical differences between adolescents with bipolar depression and healthy controls were observed. After 6 weeks of treatment with uridine, significant between-group differences were found in beta-nucleoside triphosphate (β -NTP; largely ATP) ($p = 0.008$) and phosphocreatine ($p = 0.02$).

Discussion: In this open-label case series, uridine was efficacious and well tolerated by adolescents with bipolar depression. ³¹P-MRS neuroimaging results suggested potential mediators of treatment response. Rapid onset of antidepressant action may be a distinguishing feature of uridine. While the small sample size mandates caution in interpreting these results, further study of uridine as a treatment for depressed adolescents with bipolar disorder is warranted.

Disclosure: D. Kondo: None.

198. Suboxone Use in Patients with Psychiatric Disorders

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Background: Buprenorphine is a partial opioid agonist found to be extremely in effectively treating opioid dependency and preventing the associated health consequences. Combined with naltrexone as Suboxone it is effective in blocking craving, and relative safe with low risk for dependency and diversion. It can be prescribed outside of specialized treatment programs and in physician's offices, thereby revolutionizing opioid treatment. There is little research on its use when mental disorders are comorbid.

Methods: Retrospective review of all SCID diagnosed patients with the opportunity for treatment for at least two years and who completed ratings for mood and anxiety disturbances ($n = 47$). Patients were dosed to reduce craving and withdrawal symptoms.

Results: In a simple linear regression, there was no direct relationship between comorbidity of various mental disorders and final dosage of Suboxone. There was a non-significant trend toward an increase in Suboxone dose with increased age. The score on the Mood Disordered Questionnaire showed a significant correlation with Suboxone dose ($p < 0.0001$). Patients with at least four hospitalizations in the previous year had none during the subsequent year of Suboxone treatment.

Discussion: Comorbidity affected Suboxone dose which may help to explain why some patients require amounts beyond maximum possible occupancy of the receptor. There was some evidence for additional psychotropic properties. Suboxone is safe and effective in patients with comorbid psychiatric disorders.

Disclosure: W. Lawson: Part 1; AstraZeneca, Pfizer, Reckitt Benckiser. Part 4; Astra Zeneca, Pfizer, Reckitt Benckiser. A. Cotton: None. A. Thomas: None.

199. Cannabidiol as a new type of an antipsychotic: Results from a placebo-controlled clinical trial

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Background: There is urgent need for the identification of new pharmacological targets for the treatment of schizophrenia. We recently discovered that increase of anandamide levels by blocking its degradation by administration of cannabidiol, a purified phytocannabinoid, is accompanied by significant improvement of symptoms in acute schizophrenic patients.

Methods: We performed a randomized, double-blind, placebo-controlled, cross-over clinical trial in acute, antipsychotic-naïve, first-break paranoid schizophrenia patients, fulfilling diagnostic criteria of DSM-IV. 29 patients were treated after written informed consent with either cannabidiol (600mg per day) or placebo for 14 days and then switched to the corresponding cross-over condition. Additional patients to gain a total of 18 patients treated per protocol replaced dropouts.

Results: Cannabidiol significantly improved psychotic symptoms in the cannabidiol-placebo condition during the first 14 days of treatment when compared to baseline. A MMRM analysis of all randomized patients ($n = 29$) yielded an mean improvement of 2.4 points (standard error 3.0) on PANSS total in favor of cannabidiol (vs. placebo), albeit not statistically significant. Only one patient on sequence cannabidiol-placebo terminated treatment early (last seen at visit 3) whereas 10 patients terminated early on sequence placebo-cannabidiol. The most frequent reason given was worsening of symptoms (5/11 patients).

Discussion: Although limited by design issues (cross-over), duration of treatment (14 days), and relevant placebo-response rates, this is the second study to provide evidence for antipsychotic properties of cannabidiol accompanied by a superior side-effect profile. Future placebo-controlled parallel-group trials studying the antipsychotic properties of cannabidiol in acute schizophrenia are necessary to

provide further evidence for its efficacy in the treatment of this devastating disease.

Disclosure: F. Leweke: Part 1; curantis UG (Ltd.) - Shareholder, AstraZeneca - Speakers Board, BMS - Speakers Board, Essex Pharma - Speakers Board, Servier - Speakers Board, Novartis - Consultant, Cannasat Therapeutics - Consultant. L. Kranaster: None. M. Hellmich: None. D. Koethe: AstraZeneca - Speakers Board.

200. Preclinical and Clinical Evaluation of JNJ-18038683, a Selective 5-HT₇ Receptor Antagonist, in the Physiology of Sleep and in Major Depressive Disorder

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Background: 5-HT₇ receptors are highly expressed in the thalamus and hypothalamus of both humans and rodents and have been linked to a number of psychiatric disorders including anxiety, depression, and schizophrenia. 5-HT₇ receptor blockade has been shown to be effective in animal models of depression, and, in rodents, to increase the latency to REM sleep and decrease REM density. In humans, major depressive disorder is often characterized by a short latency to REM sleep and an increased REM density. Thus, we hypothesized that increasing REM latency and decreasing REM density in humans might have antidepressant effects. To the best of our knowledge, no systematic clinical evaluation of the effectiveness of 5-HT₇ blockade has been conducted.

Methods: We report here, for the first time, the preclinical and clinical evaluation of a selective 5-HT₇ receptor antagonist, JNJ-18038683.

Results: JNJ-18038683 increased the latency to REM sleep and decreased REM density in rodents, and was also effective in standard behavioral models of depression such as the mouse tail suspension test. In addition, JNJ-18038683 enhanced 5-HT transmission, antidepressant-like behavior, and REM suppression induced by citalopram in rodents. JNJ-18038683 also increased the latency to REM sleep and decreased REM density in humans. Effects of JNJ-18038683 in depressed patients were equivocal due to a high placebo rate.

Discussion: These results show that the physiological effects of 5-HT₇ receptor blockade translate from rodents to humans and that further clinical studies are warranted to evaluate the clinical potential of this class of agents in major depressive disorder.

Disclosure: T. Lovenberg: Part 5; Johnson & Johnson Pharmaceutical R&D, LLC. P. Bonaventure: Johnson & Johnson Pharmaceutical R&D, LLC. M. Kramer: Johnson & Johnson Pharmaceutical R&D, LLC. S. Sands: Johnson & Johnson Pharmaceutical R&D, LLC. P. De Boer: Johnson & Johnson Pharmaceutical R&D, LLC. C. Dugovic: Johnson & Johnson Pharmaceutical R&D, LLC. H. Manji: Johnson & Johnson Pharmaceutical R&D, LLC.

201. The Corticotropin-Releasing Factor (CRF)₁ Receptor Antagonist GW876008 Inhibits Activation and Connectivity of an Emotional Arousal Circuit in Patients with Irritable Bowel Syndrome and Comorbid Anxiety

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Background: Based on extensive preclinical data, alterations in the activity of the central CRF/CRF₁ receptor signaling system have been implicated as a possible mechanism in the pathophysiology of anxiety, depression and stress sensitive pain disorders, such as Irritable Bowel Syndrome (IBS) (Bradesi & Mayer, 2007) Visceral hyperalgesia, increased defecation, and anxiety-like behavior in rodent models of early life, as well as acute and chronic adult stress are attenuated by CRF₁ receptor antagonists (Martinez & Tache, 2006). In the present

study, we aimed to determine the effect of a selective CRF₁ receptor antagonist, GW876008 (20 and 200 mg), compared to placebo, on brain activity within a stress-related emotional-arousal circuit.

Methods: Regional brain responses to a pain threat protocol were studied in 28 female subjects (16 healthy controls, 12 with a diagnosis of IBS) using functional magnetic resonance imaging (fMRI). The protocol consisted of THREAT and SAFE conditions wherein subjects were instructed they may or may not receive an electrical shock to the left lower abdomen, respectively. A 2 (Group IBS; HC) x 3 (Drug: placebo; 20 mg and 200 mg GW876008) general linear model specifying subject as a random effect and controlling for order was applied to test treatment effects on response to threat for anatomically defined region of interests (ROIs) using contrast beta images representing signal changes between stimulus conditions (THREAT - SAFE). In addition, effective connectivity between nodes of an emotional-arousal circuit (including amygdala [AMYG], anterior insula [aINS], anterior cingulate cortical [ACC] subregions, orbitofrontal cortex [OFC], locus coeruleus complex [LCC], hypothalamus [HT], and hippocampus [HC]) was determined using structural equation modeling (Labus et al, 2008).

Results: Acute administration of GW876008 (20 mg, or 200 mg dose) compared to placebo resulted in significant reductions in fMRI blood oxygen level-dependent (BOLD) responses in the left AMYG and OFC, and bilateral subgenual ACC. In addition, significant Group x Drug interactions were found for the left LCC and left HT. During placebo, IBS patients showed greater BOLD responses to the pain threat in the left LCC and HT during the threat of abdominal pain task, compared to HCs. Administration of GW876008 (both doses) resulted in a significant reduction in activity in the left HT in IBS patients, but not in HCs. Significant Group x Drug x State Anxiety interactions were observed for the left HT: For average and high state (but not low) anxiety, patients compared to HCs showed greater BOLD signal response in the left HT following placebo, and greater reductions in BOLD responses during 20 mg and 200 mg doses of GW876008, compared to placebo. Effective connectivity analysis showed a significant drug induced increase in connectivity for paths to the AMYG from OFC, HPC, and HT, approaching levels seen in HCs during placebo for the latter two regions. In addition, IBS showed drug induced reductions in connectivity from rostral ACC and LCC to AMYG, and from AMYG to aINS.

Discussion: Acute administration of GW876008 was associated with significantly greater reductions in BOLD responses to a threat of pain in AMYG, LCC, subgenual ACC and HT compared to placebo. Drug administration also resulted in normalization of effective connectivity between these regions. When viewed together, these findings are consistent with the hypothesized effect of CRF₁ receptor antagonism on key regions of and activity within a stress-related emotional-arousal network, expected from preclinical studies. The inhibitory effects were contingent on the presence of average and high state anxiety, since they were not seen in subjects with low state anxiety. Consistent with preclinical models, these results suggest a potential pathway for therapeutic benefit of CRF₁ receptor antagonism for stress-sensitive disorders, including anxiety disorders and irritable bowel syndrome.

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202. Clomipramine and Quetiapine Augmentation for Obsessive Compulsive Disorder Compared with Sustained Fluoxetine Treatment

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Background: Obsessive-compulsive disorder (OCD) first line treatments have a partial [or no] effect for up to 40% of patients.

Augmentation strategies with several pharmacological agents have been tried in many open and a few double-blind controlled trials. Current evidence supports the augmentation of serotonin reuptake inhibitors (SSRI) with typical and atypical anti-psychotics. However, anti-psychotics have been associated with severe side effects which lead to higher cardio-vascular morbidity in the long term. Therefore, new augmentation strategies are needed to avoid such adverse events. Augmentation of SSRI with other compounds has yielded positive responses in some open and a few double-blind clinical trials. However, so far, the efficacy of these strategies has not been compared with that obtained with the association of anti-psychotics with SSRI.

Methods: Previously to the beginning of this trial all patients had to report OCD as they primary diagnosis, be taking the highest tolerate or recommended dosage of fluoxetine for at least 8 weeks, have a current Yale Brown Obsessive-Compulsive Scale (YBOCS) score of at least 16, and have had a reduction of less than 35% of the initial YBOCS score with fluoxetine treatment. Potential risk of complication associated with the medication used in this trial (e.g. baseline EKG alteration), age 65, current pregnancy or lactation, mania, eminent suicidal risk, psychotic symptoms or drug dependence were used as exclusion criteria. Sixty-nine OCD patients who met these inclusion criteria were invited to participate in a randomized double blind trial with three arms: Sustained high-dose fluoxetine (up to 80 mg/day) plus placebo (FLX), reduced dose fluoxetine (up to 40 mg/day) plus clomipramine (up to 75 mg/day) (CLM) and reduced dose fluoxetine (up to 40 mg/day) plus quetiapine (up to 200 mg/day) (QTP). Fifteen patients refused to participate. Eighteen patients (N=54) were allocated in each arm with a minimization procedure. Blinded raters, not involved in patients' care, collected YBOCS scores at weeks 0 and 12. Intermediate measures (weeks 1,2,3,4 and 8) were performed by a psychiatrist during routine consultation. Sample characteristics were: mean current age of 34 years, mean age at OCD onset of 12 and mean initial YBOCS score of 25. Thirty-two patients (59%) reported current depressive episode, five patients (9%) met criteria for Tourette's syndrome and other six (11%) for chronic tic disorder. Forty-three patients (80%) had reached maximum fluoxetine dosage (80 mg/day) at first treatment with a mean YBOCS reduction of 27%. During the augmentation phase patients were submitted to periodic EKG evaluation and fluoxetine and clomipramine serum dosage. Analyses were made with intention-to-treat and hot-deck imputation of missing data. Wilcoxon Signed Ranks Test was used to compare endpoint with baseline measures. Kruskal-Wallis test was used for comparison between groups.

Results: Forty-one patients (76%) completed twelve weeks follow-up. Seven patients (2 CLM, 3 QTP, 2 FLX) abandoned treatment; three patients (3 QTP) did not tolerate side effects; and four (3 CLM, 1 QTP) discontinued due to perceived clinical risk. No severe adverse events occurred during the trial. FLX and CLM groups were significantly better compared to baseline measures after 12 weeks of treatment. FLX and CLM were very similar regarding response measures, while the QTP group was significantly worse ($p = 0.001$). Sensitivity analyses showed that other imputation methods (last observation carried forward, worst case scenario) did not significantly change results.

Discussion: This is the first trial that evaluated clomipramine augmentation in a double-blind placebo controlled format. As well, it is the first to compare quetiapine augmentation with another active augmentation. Clomipramine did not show superiority to sustained high dosage fluoxetine plus placebo and was not associated with severe adverse events in this trial. Quetiapine did not significantly change initial YBOCS scores and was associated with lower treatment response compared with the other two groups. Previous trials had found inconsistent results regarding treatment response with quetiapine augmentation and clomipramine had been evaluated only in fairly small uncontrolled trials. Limitations of our trial include the use of low dosage of augmenters, differential drop-out rates for each treatment arm and short period of follow-up. Despite these limitations, our results support the prorogation of the period of maximum dosage of SSRIs before pharmacological augmentation is tried.

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203. Subcortical/Cortical Effects of Single-Dose Modafinil During Cognitive Control Performance in Schizophrenia

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Background: Cognitive deficits are a core feature of illness in schizophrenia; they predate overt illness onset, persist during periods of symptom remission, and are strong predictors of long-term functional outcome. Critically, they are not effectively treated with the current antipsychotic pharmacopoeia. We used the combined NET/DAT inhibitor modafinil to test whether modulation of catecholamine systems is effective to remediate the altered function of the cortical/subcortical cognitive control network in schizophrenia.

Methods: 27 clinically-stable patients with DSM-IV-TR-defined schizophrenia participated in a randomized, double-blind, counterbalanced, placebo-controlled within-subjects study of modafinil, administered as a single oral 200 mg dose. Patients were scanned on a 3T MRI while they performed the Preparing to Overcome Prepotency (POP) Task, a cued visual stimulus-response mapping task requiring proactive control, with a slow event-related design. Results were evaluated within this group, and compared to a healthy control group who participated in the identical protocol and data analysis procedures.

Results: The patient group was faster and more accurate on drug versus placebo. The patient group showed significant task-independent deactivation in subcortical regions including the locus coeruleus (LC) and the substantia nigra/ventral tegmental area (SN/VTA), and some evidence of increased prefrontal cortex (PFC) activity in a Treatment-by-Task Condition contrast. When directly compared to the control group, the patient group showed A) excessive cue-independent deactivation in VTA and striatum; B) attenuated cue-independent deactivation in LC; C) impaired Treatment-by-Condition increases in both VTA and LC to the Cue; D) greater Treatment-by-Condition increases in VTA to the Probe; E) a varied set of increases and decreases throughout the PFC, as a function of Treatment-by-Condition in the patient group relative to controls, both to Cue and Probe.

Discussion: Among schizophrenia patients, modafinil modulates the subcortical catecholamine nuclei that modulate the PFC. While these effects coincide with increased Treatment-by-Condition activity in the PFC (suggestive of enhanced gain control) and enhanced performance, they are altered compared to healthy controls. The pattern of altered effects on catecholamine nuclei are consistent with the underlying effects of chronic antipsychotic treatment on catecholamine neuron firing. Further work should directly address this problem to establish the optimal conditions for pro-cognitive drug action, and evaluate the relative benefits of single-dose versus sustained modafinil treatment for brain and cognition in schizophrenia.

Disclosure: M. Minzenberg: None. J. Yoon: None. J. Nunez del Prado: None. S. Soosman: None. C. Carter: None.

204. Biobehavioral Mechanisms of Naltexone's Action on Drinking in Adolescents: Preliminary Findings

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Background: An estimated 1.4 million teenagers meet criteria for an alcohol use disorder (AUD) in the U.S. each year, yet only 16% receive treatment and outcome research indicates that many do not benefit

from psychosocial interventions alone. Although considerable advances have been made in the development of pharmacotherapy for adults with AUDs, double-blind, placebo-controlled clinical trials with adolescents are almost nonexistent and published trials all bear substantial limitations that preclude inferences about the efficacy of the medication studied. This gap in knowledge impedes adolescent treatment practices, as compelling evidence indicates the safety and efficacy of medication use with youth cannot be inferred from adult data. The major objective of this ongoing study is to test the effects of naltrexone (NTX) - one of the most efficacious medications for treating AUD in adults - on craving and the acute subjective effects of alcohol ingestion among adolescents.

Methods: Non-treatment seeking adolescent drinkers ($n=15$; ages 15-18 years) were enrolled in an ongoing double-blind, placebo-controlled, within-subjects study. Participants received NTX (50 mg/day) and placebo for 10 days in counterbalanced order, with at least a 4-day washout period between each arm of the study. To assess craving and subjective effects of alcohol in participants' natural environments, ecological momentary assessment (EMA) was conducted by having teens use handheld electronic diaries to monitor their drinking, mood, urge to drink, and subjective effects of alcohol ingestion in real time for each 10-day period. At the end of each 10-day period, participants completed a laboratory-based alcohol cue reactivity assessment to investigate the effects of NTX on cue-elicited craving.

Results: Medication and EMA compliance was high across the trial. NTX was well tolerated by youth. Preliminary analyses of NTX's effects on alcohol cue reactivity in the laboratory were conducted using a 2 Cue Type (water, alcohol) X 2 Medication Condition (NTX, placebo) repeated measures ANOVA. Results show alcohol cues elicit robust increases in subjective craving among adolescents ($F [1, 14] = 9.58$, $p < .01$, $\eta^2 = .41$); and that NTX reduces craving for alcohol across both cue types ($F [1, 14] = 4.08$, $p = .06$, $\eta^2 = .23$); the interaction between Cue Type and Medication Condition was not significant. EMA data collected in participants' natural environments similarly show that NTX reduces craving ($p = .08$). In addition, as seen in adult samples, EMA data indicate that NTX altered a number of the subjective effects of acute alcohol ingestion ($ps < .05$), but the directionality of these effects was mixed compared to those observed in adults.

Discussion: This study provides important new information about the mechanisms of NTX action in an understudied population at high risk for developing lasting alcohol-related problems. In addition, this project establishes the utility of a comprehensive yet efficient paradigm for studying drinking and mechanisms of pharmacotherapy action in youth, a population for whom alcohol administration is precluded for ethical reasons. Initial analyses of this ongoing study provide preliminary evidence that NTX reduces craving and alters the subjective effects of alcohol in adolescent drinkers. These findings suggest common mechanisms may underlie alcohol craving in adults and adolescents. Research supported by NIAAA (AA017273; PI: R. Miranda, Jr.).

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205. The Combination of Metyrapone and Oxazepam Reduces Intravenous Nicotine Self-Administration in Rats

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Background: Although recent pharmacological advances have increased rates of smoking cessation, current pharmacological interventions have relatively low levels of efficacy and are associated with significant adverse events. Over the past several years, our lab, as well

as a number of others, has investigated the complex relationship between stress and addiction. We have studied a number of compounds that affect the stress response, with a particular focus on the HPA axis, and looked in detail at their ability to affect cocaine and methamphetamine self-administration in rat models. We have previously reported that combinations of metyrapone and oxazepam, two compounds that affect the stress response through different mechanisms, administered at doses that were ineffective when delivered singly, resulted in dose-related decreases in cocaine self-administration in rats. The doses tested did not affect food-maintained responding during the same sessions. The current study was designed to test the same combination during intravenous nicotine self-administration in rats.

Methods: Rats were implanted with chronic indwelling jugular catheters and were trained to self-administer nicotine (0.03 mg/kg/infusion) during daily 1-hour self-administration sessions under a fixed-ratio 1, timeout 20 sec (FR1-TO-20s) schedule of reinforcement. When stable baselines of responding were obtained, the rats were injected intraperitoneally (ip) with vehicle (5% Alkamuls EL-620 in 0.9% saline) and three dose combinations of metyrapone and oxazepam (metyrapone:oxazepam; 25:5, 50:5, and 50:10 mg/kg) or subcutaneously with the positive control varenicline (1.0 mg/kg). Following testing under a FR1-TO-20 s schedule of reinforcement, rats were tested under a progressive-ratio (PR) schedule of reinforcement with the lowest metyrapone:oxazepam dose combination (i.e., 25:5 mg/kg, ip).

Results: The administration of a combination of low doses of metyrapone and oxazepam produced dose-related decreases in intravenous nicotine self-administration in rats, with significant reductions observed with all dose combinations. The effect of the highest dose combination tested (i.e., metyrapone:oxazepam, 50:10 mg/kg) was significantly greater than that of varenicline. When rats were tested under the PR schedule of reinforcement, the lowest dose combination (i.e., metyrapone:oxazepam, 25:5 mg/kg) significantly reduced the number of nicotine infusions and the corresponding "breakpoint" compared to vehicle-treated controls.

Discussion: Combinations of low doses of metyrapone and oxazepam decreased intravenous nicotine self-administration in rats in a dose-related manner. Significant reductions in nicotine infusions were even seen with the lowest dose combination tested, suggesting that nicotine self-administration may be even more sensitive to the effects of the combination relative to cocaine self-administration. Although the single agents were not tested in the current study, the doses of metyrapone and oxazepam chosen for testing as a combination have no effect on food self-administration using similar operant techniques, suggesting that the effects on nicotine were not the result of nonspecific effects on the ability of the rats to lever press. The lowest dose combination of metyrapone and oxazepam also reduced nicotine self-administration maintained under a progressive-ratio schedule of reinforcement, suggesting that the combination of metyrapone and oxazepam reduces the motivation to seek and take nicotine. Finally, the effects of the combination of metyrapone and oxazepam were greater than those of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline. Since varenicline is effective in reducing cigarette smoking in humans and appears to be more beneficial than either bupropion or nicotine replacement therapy, these data suggest that the combination of metyrapone and oxazepam may also be effective in reducing smoking.

Disclosure: N. Goeders: *Part 1*; Embera NeuroTherapeutics, Inc. *Part 2*; Embera NeuroTherapeutics, Inc. *Part 3*; Embera NeuroTherapeutics, Inc. *Part 4*; Embera NeuroTherapeutics, Inc. B. Fox: *Part 1*; Embera NeuroTherapeutics, Inc. *Part 4*; Embera NeuroTherapeutics, Inc. M. Azar: *Part 1*; Embera NeuroTherapeutics, Inc. *Part 4*; Embera NeuroTherapeutics, Inc. *Part 5*; Behavioral Pharma. B. McCarthy: *Part 1*; Embera NeuroTherapeutics, Inc. *Part 3*; Embera NeuroTherapeutics, Inc. *Part 5*; Afferent Pharmaceuticals. G. Koob: *Part 1*; Embera NeuroTherapeutics, Inc. *Part 4*; Embera NeuroTherapeutics, Inc.

206. Social Withdrawal in the PCP Rat Model of Schizophrenia Involves Reduced Cannabinoid CB₁ Receptor Stimulation

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Background: Withdrawal from repeated phencyclidine (PCP) administration reduces social interaction in rats, a behavioral phenotype that mimicks the negative symptoms of schizophrenia. Previous studies have shown that the endocannabinoid system may be involved in the pathophysiology of schizophrenia and that CSF levels of the endocannabinoid anandamide (AEA) are inversely correlated with negative symptoms in drug-naïve schizophrenic patients. In agreement with these observations, we found that pharmacological enhancement of AEA tone by URB597, an inhibitor of AEA degradation, improved social interaction in rats undergoing withdrawal from sub-chronic PCP (5 mg/kg, i.p., twice daily for 7 days) in a CB₁-dependent manner. These studies suggest that PCP-induced social withdrawal is associated with reduced AEA activity at cannabinoid CB₁ receptors.

Methods: To test this hypothesis, we investigated whether pharmacological blockade of CB₁ receptors by the CB₁ antagonists AM251 (0.3-3.0 mg/kg, i.p.) and SR141716A (0.1-1.0 mg/kg, i.p.) reduced social interaction (i.e., sniffing, following and climbing) in normal rats (Albino Wistar rats, 250-300 g, Charles Rivers). We also studied whether the potent cannabinoid agonist CP55,940 (0.01 mg/kg, i.p.) could reverse PCP-induced social withdrawal, similarly to URB597. Finally, to assess AEA transmission, two independent groups of rats (saline- and PCP-treated) were sacrificed immediately after behavioral assessment to measure AEA levels by mass spectrometry in brain areas relevant to schizophrenia.

Results: In normal rats, systemic administration of the CB₁ antagonists AM251 or SR141716A dose-dependently reduced the time spent in social interaction, thus producing a deficit similar to that observed in PCP-treated rats. In addition, administration of the cannabinoid agonist CP55,940 reversed PCP-induced social withdrawal in a CB₁-dependent manner without altering social interaction in control rats. In agreement with our hypothesis, PCP-induced social withdrawal was accompanied by significantly decreased levels of AEA in the amygdala and prefrontal cortex, two brain regions known to regulate social behaviors.

Discussion: Taken together, these findings indicate that reduced AEA activity at CB₁ receptors underlies PCP-induced social withdrawal and that elevation of endocannabinoid tone could be beneficial for the treatment of the negative symptoms of schizophrenia.

Disclosure: A. Seillier: None. A. Martinez: None. A. Giuffrida: None.

207. Trace Amine-Associated Receptor 1 (TAAR1)-Mediated Effects of d-Methamphetamine

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Background: Illicit methamphetamine (METH) consumption is declining nation-wide yet significant use persists in specific demographics of the United States in spite of overwhelmingly negative personal, familial and legal consequences associated with its use and abuse. For more than 70 years the substrates mediating METH's abuse potential have been investigated. In spite of clarifying some of the mechanisms underlying METH's actions (e.g. interference with dopamine, norepinephrine and vesicular monoamine transporter functions as well as monoamine oxidase activity) there is still no widely accepted pharmacologic approach to managing the METH abstinence syndrome or preventing relapse to its abuse. The lack of

progress in this area suggests important biological molecules that mediate METH's abuse liability other than transporters and enzymes remain to be discovered, a view supported by research involving genetically engineered mice. Previously we reported recombinant mouse, rat and human GalphaS-coupled Trace Amine-Associated Receptor-1 (TAAR1) is activated *in vitro* by nanomolar concentrations of d-METH to stimulate cAMP production. However, the extent to which TAAR1 contributes to the behavioral and/or physiological effects elicited by acute d-METH exposure has yet to be determined. Using a novel approach we resynthesized in-house the recently reported first TAAR1-selective antagonist N-(3-ethoxy-phenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide (EPPTB) and are the first to report its use to directly assess TAAR1-mediated effects of d-METH *in vitro* and *in vivo*.

Methods: A novel 5-step route to the synthesis of EPPTB was developed. BOC-protected 4-amino-5-trifluoromethyl benzoic acid was coupled to 3-ethoxybenzylamine and the product was then reacted with tetrahydro-2,5-dimethoxyfuran under highly acidic conditions. The resulting oily EPPTB was deprotected and converted to its HCl salt. HEK293 cells stably expressing recombinant mouse TAAR1 (mTAAR1) or harboring the empty expression vector were grown under standard conditions to ~ 80% confluence. Cells were harvested, exposed to drug or vehicle and cAMP content was measured in extracts using the DiscoverX HitHunter assay. For the behavioral study adult male wild type C57Bl/6J mice were assigned to four experimental groups. Every 24 hours body weight, core temperature, food and water consumption were recorded. A Topscan video monitoring system was used to document animal activity. On day 1 mice were handled and individually placed in an open field for 90 minutes. On day 2 all mice were given intraperitoneal (i.p.) injections of vehicle at t = 0, placed in an open field for 30 minutes, given another injection of vehicle and monitored for an additional 60 minutes. On day 3 group 1 mice received injections of vehicle at t = 0 and t = 30; group 2 mice an injection of vehicle at t = 0 and 5 mg/kg METH at t = 30; group 3 mice were injected with 11 mg/kg EPPTB at t = 0 and vehicle at t = 30; group 4 mice were injected with EPPTB at t = 0 and METH at t = 30. On day 4 all mice received vehicle injections at t = 0 and t = 30. Data were evaluated by 2-way ANOVA with drug and day as main factors followed by Bonferroni's post-test. A p < 0.05 was considered significant.

Results: Our novel approach to synthesizing EPPTB had a yield of ~ 30%. The composition, structure and purity of our EPPTB was confirmed by NMR and mass spectrometry. *In vitro* our EPPTB completely blocked both phenylethylamine- (PEA) and d-METH-stimulated cAMP production in HEK293 cells expressing mTAAR1 with pIC₈₀'s of ~100 nM. In the absence of agonist EPPTB suppressed basal cAMP levels in mTAAR1 expressing cells. Neither EPPTB, PEA or METH had any effect on cAMP levels in HEK293 cells harboring the empty expression vector. Schild plot analyses revealed EPPTB blocked PEA- and METH-stimulated cAMP production in a manner consistent with it being a competitive antagonist, although high concentrations of METH were better able than PEA to overcome its antagonism. Confident our in-house synthesized EPPTB was antagonizing METH-stimulated mTAAR1-mediated cAMP production *in vitro* we have initiated an *in vitro* electrophysiological study as well as an *in vivo* evaluation of its effects in the absence and presence of an acute dose of METH.

Discussion: Our results are the first to conclusively demonstrate METH stimulated mTAAR1-mediated cAMP production in HEK293 cells is competitively antagonized by EPPTB. These results are consistent with our hypothesis TAAR1 plays a role in METH's abuse liability but demonstrating EPPTB blocks an *in vivo* effect of METH will provide and even more compelling justification for pursuing TAAR1 as a medication target.

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208. Raclopride Lessens the Ability of Clozapine to Suppress Alcohol Drinking in Syrian Golden Hamsters

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Background: Patients with schizophrenia commonly develop alcohol use disorders, characterized by regular consumption of modest amounts of alcohol; even this modest alcohol use, however, dramatically worsens the course of schizophrenia. Most antipsychotic medications do not diminish alcohol or substance abuse; however, our preliminary data suggest that the atypical antipsychotic clozapine (CLOZ) does. Unlike typical antipsychotics, all of which have potent dopamine (DA) D₂ receptor blocking ability, CLOZ is a broad spectrum agent with relatively weak affinity for the DA D₂ receptor. Regarding the effects of antipsychotics, we have proposed that: (a) patients with schizophrenia have a reward deficiency syndrome, secondary to a dysfunction in the mesocorticolimbic DA circuitry, that underlies alcohol use in this population; (b) most antipsychotic drugs do not decrease alcohol use in this population largely because they do not restore the normal function of the DA-mediated mesocorticolimbic DA reward pathways (in part because of their potent D₂ receptor blocking effect); and (c) CLOZ, through its various actions on multiple neurotransmitter systems, particularly its potent blockade of α -2 noradrenergic receptors, its striking increase in norepinephrine (NE) levels, as well as its weak blockade of dopamine D₂ receptors, may tend to have a normalizing effect on the dysfunctional mesocorticolimbic brain reward circuit. The purposes of the current study were: (1) to further assess the effect of clozapine on alcohol consumption in hamsters; and (2) to examine whether CLOZ's effect on alcohol intake is related to its weak DA D₂ receptor blockade. To achieve these goals, we assessed the ability of the potent DA D₂ receptor antagonist raclopride (RACL) to diminish the suppressive effect of CLOZ on alcohol intake in the hamster.

Methods: In Experiment 1, to assess the effects of CLOZ and RACL on initiation of alcohol intake, hamsters were individually housed with free access to water and food. After a baseline period, hamsters were treated with daily, subcutaneous (s.c.) injections of: 2 mg/kg clozapine; 4 mg/kg clozapine; 2 mg/kg raclopride; 2 mg/kg clozapine + 2 mg/kg raclopride; 4 mg/kg clozapine + 2 mg/kg raclopride; or vehicle for 18 days. In addition, to maintain high levels of DA D₂ blockade during the hamsters' active phase, 2 mg/kg raclopride (or vehicle) was given in a second daily injection (6 hours after the first). Four days into treatment, hamsters were given free access to a bottle containing a 15% alcohol solution, which remained freely available for the duration of treatment. In Experiment 2, hamsters were individually housed with free access to 15% alcohol, water and food. After a baseline period, hamsters were treated with daily, s.c. injections of: 2 mg/kg raclopride; 4 mg/kg raclopride; 6 mg/kg raclopride; 4 mg/kg clozapine; 4 mg/kg clozapine + 2 mg/kg raclopride; 4 mg/kg clozapine + 4 mg/kg raclopride; 4 mg/kg clozapine + 6 mg/kg raclopride; or vehicle for 20 days.

Results: In Experiment 1, clozapine (2 and 4 mg/kg) reduced the acquired baseline alcohol intake levels by about 25%, relative to vehicle treatment. This reduction of baseline alcohol intake was substantially less than the reduction of chronic alcohol intake seen in Experiment 2. In Experiment 2, clozapine treatment was initiated in hamsters that had already established stable baseline alcohol intake. Clozapine (4 mg/kg) decreased chronic alcohol intake by about 45%. In Experiment 1, raclopride (2 mg/kg, given twice a day) essentially reversed the effect of 2 mg/kg clozapine on initiation of alcohol intake. However, the same dose of raclopride only partially reversed the effect of 4 mg/kg clozapine. In Experiment 2, while clozapine alone decreased ongoing alcohol drinking more, adding raclopride (either 2 or 6 mg/kg) to clozapine (4 mg/kg) lessened the suppression of alcohol drinking by clozapine by approximately 30%. The lowest dose (2 mg/kg) and the highest dose (6 mg/kg) of raclopride were equally

effective in lessening the effects of clozapine, while the middle dose (4 mg/kg) was ineffective.

Discussion: The major findings were: (1) clozapine suppressed both initiation and maintenance of alcohol intake in hamsters; and (2) these effects of clozapine were lessened when raclopride, a potent DA D₂ receptor antagonist, was given adjunctively with clozapine. These data suggest that clozapine may limit alcohol intake in the golden hamster (and possibly in patients with schizophrenia) in part because of its weak blockade of the DA D₂ receptor.

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209. Effects of Methylphenidate Administration on Striatonigral Neurotensin Systems: A Comparison with Cocaine and Methamphetamine Treatment

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Background: Methylphenidate (Ritalin) is an effective stimulant in the treatment of attention deficit hyperactivity disorders. Mechanistically, methylphenidate (MPD) is a dopamine (DA) reuptake inhibitor that induces DA overflow in the striatum by binding to the DA transporter in a manner similar to cocaine (COC). Since previous studies demonstrated that psychostimulants, such as cocaine (Hanson et al., 1989) and methamphetamine (METH; Letter et al., 1987) differentially alter rat brain neurotensin (NT) systems through DA mechanisms, the present study evaluated the effects of MPD on striatal and nigral neurotensin-like immunoreactivity (NTLI) and compared the underlying DA mechanisms on MPD-induced changes in striatal and nigral NTLI content with other psychostimulants.

Methods: Sprague-Dawley rats received multiple administration of MPD (10.0 mg/kg, s.c.) in the presence or absence of selective DA receptor antagonists (D₁; SCH23390 or D₂; eticlopride), and were killed 18 h after drug treatment. The NTLI was determined by employing a highly selective RIA analysis.

Results: MPD treatment profoundly affected NT systems by increasing both striatal and nigral NTLI content; these increases in NTLI were blocked by coadministration of either D₁ or D₂ receptor antagonists. In the striatum, these blockade patterns were similar to those produced by COC, but differed from METH effects, which were only blocked by the D₁, but not the D₂ antagonist. A similar pattern was observed in the substantia nigra; thus the MPD- and COC-induced increases in NTLI that were completely or partially prevented by the action of D₁ or D₂ antagonists, while the METH-mediated increases in NTLI were only antagonized by the D₁ receptor blocker.

Discussion: In summary, basal ganglia NT is significantly affected by MPD through DA mechanisms that appear more like COC than METH. This work was supported by U.S. Public Health Service Grants DA09407, DA00378, DA11389 and DA019447.

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210. Psychostimulant-Induced Hyperlocomotion and Stress-Induced Hyperthermia Are Blocked by a TAAR₁ Agonist via Modulation of Monoaminergic Activity

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Background: The trace amine-associated receptor 1 (TAAR₁) is a Gs-type G protein coupled receptor that is activated by endogenous metabolites of amino acids, like the trace amines p-tyramine,

beta-phenylethylamine, octopamine, and tryptamine. TAAR1 is considered a promising drug target for the treatment of psychiatric and neurodegenerative disorders. Previous studies have shown that TAAR1 knock-out mice are hypersensitive to the effects of amphetamine, with enhanced locomotor activity and increased striatal release of dopamine, serotonin and noradrenaline, strongly suggesting that TAAR1 is an important modulator of the monoaminergic system. Use of the first TAAR1 antagonist EPPTB further demonstrated that TAAR1 tonically activates inwardly rectifying K⁺ channels, which reduce the basal firing frequency of dopaminergic neurons in the ventral tegmental area. Herein is described the effects of RO5166017, the first selective TAAR1 agonist showing *in vivo* activity. The compound will help to better understand the role of TAAR1 in the control of monoaminergic-mediated functions and behaviors.

Methods: RO5166017 was evaluated for its binding affinity and functional activity at various receptors stably expressed in HEK293 cells, for its effects on the firing frequency of monoaminergic neurons, and for its *in vivo* properties in modulating stress-induced hyperthermia or psychostimulant-induced hyperlocomotion in mice.

Results: RO5166017 showed high affinity and potent functional activity at mouse, rat, cynomolgus and human TAAR1 stably expressed in HEK293 cells as well as high selectivity versus other receptors, transporters and enzymes. In mouse brain slices RO5166017 inhibited the firing frequency of dopaminergic and serotonergic neurons in regions where TAAR1 is expressed, i.e. the ventral tegmental area and dorsal raphe nucleus, respectively. In contrast, RO5166017 did not change the firing frequency of noradrenergic neurons in the locus coeruleus, an area devoid of *Taar1* expression. RO5166017 has good pharmacokinetic properties. *In vivo*, the compound prevented the development of stress-induced hyperthermia (at 0.1 and 0.3 mg/kg p.o.), and of cocaine-induced hyperlocomotion (from 0.03 to 3 mg/kg p.o.). These effects were not seen in TAAR1 knock-out mice suggesting that the anxiolytic- as well as antipsychotic-like effects of RO5166017 are TAAR1-mediated.

Discussion: The results provide further evidence for a close interaction between TAAR1 and the dopaminergic and serotonergic systems. In addition, the TAAR1 agonist was effective in behavioral procedures that detect antianxiety and antipsychotic potential, suggesting that there may be new treatment opportunities for psychiatric disorders.

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211. Use of Transgenic Mice to Investigate the Role of Hypocretin-1 (Orexin-1) Receptors in Nicotine Reinforcement

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Background: Hypocretin (or orexin) transmission plays a key role in motivated behavior for drugs of abuse. Indeed, previous studies from our laboratory and others have shown that the hypocretin-1 antagonist SB-334867 attenuates nicotine-induced lowering of brain-stimulation reward thresholds and decreases intravenous nicotine self-administration in rats. While SB-334867 certainly antagonizes the hypocretin-1 (Hcrt-1) receptor, it is derived from a class of compounds that has affinity for other non-hypocretin receptor classes as well. This raises the possibility that its effects may partly derive from "off-target" actions at non-Hcrt-1 receptors in the brain.

Methods: To unambiguously verify a role for Hcrt-1 receptors in nicotine reinforcement, we assessed nicotine self-administration behavior in male wildtype (WT) and Hcrt-1 receptor knockout (KO) mice. Following consistent operant responding for food (20 mg pellet;

FR5TO20; ~14 d), mice were implanted with a catheter into the jugular vein. Following surgical recovery (1 wk), mice self-administered nicotine during daily 1 hr sessions. In a separate cohort of mice, extinction sessions commenced following nicotine self-administration where lever press responding had no programmed consequences (10 d). After extinction, animals received a single reinstatement session where a nicotine-paired cue light was presented (20 sec) and subsequent operant responding resulted in cue light presentation. SB-334867 (Tocris) was administered IP 15 min prior to the self-administration session in all experiments.

Results: We found that the KO mice consumed significantly less nicotine across a wide range of nicotine doses (0-0.4 mg/kg/inf) compared with WT animals. This effect was not secondary to deficits in behavioral performance as WT and KO mice responded at the same high rates for food reinforcement. We also found that SB-334867 (0-4 mg/kg IP) decreased nicotine self-administration (0.1 mg/kg/inf) in WT mice, but did not further decrease nicotine intake in KO mice. Finally, we found that a nicotine-paired cue light robustly reinstated extinguished nicotine seeking responses in WT but not KO mice.

Discussion: These data demonstrate that Hcrt-1 receptors are critical for maintaining sensitivity to the reinforcing effects of nicotine and are necessary for the expression of relapse-like behavior. In addition, these data provide strong behavioral evidence that SB-334867 acts selectively at Hcrt-1 receptors *in vivo* (as oppose to the myriad other receptors in brain). Moreover, our findings highlight the potential utility of Hcrt-1 receptor antagonists for the treatment of tobacco dependence, and report a novel behavioral procedure that can support the identification of selective Hcrt-1 receptor antagonists for tobacco dependence.

Disclosure: J. Hollander: None. P. Kenny: None.

212. 5-HT₇ Receptor Antagonism Contributes to the Ability of Atypical Antipsychotic Drugs (APDs), Including Lurasidone and Amisulpride, to Reverse the Phencyclidine (PCP)-induced Deficit in Novel Object Recognition in Rats

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Background: Hypoglutamatergic cortical function is believed to contribute to cognitive deficits in schizophrenia. Sub-chronic treatment of rodents with the NMDA receptor non-competitive antagonist, phencyclidine (PCP), has been postulated to model this deficit, e.g. by inducing prolonged deficits in novel object recognition (NOR), which may be analogous to deficits in declarative memory in schizophrenia. We have reported that 5-HT_{2A/D₂} atypical (e.g. clozapine), but not typical antipsychotic drugs (APDs), reverse the subchronic PCP-induced deficit in NOR, and that 5-HT_{2A} antagonists, e.g. pimavanserin, and the mGluR2/3 agonist, LY379268, can potentiate the ability of sub-effective doses of 5-HT_{2A/D₂} atypical APDs, e.g. lurasidone, to ameliorate the PCP-induced NOR deficit (Snigdha et al. 2010, Horiguchi et al. submitted). It has been suggested that 5-HT₇ agonism may also be an effective target for improvement of cognitive impairment (Perez-Garcia and Meneses, 2005). Consistent with this, a study of a high dose of the 5-HT₇ antagonist SB269970 on NOR in rats reported impairment in NOR (Ballaz et al. 2007). We have now tested the ability of SB269970 alone, or in combination with lurasidone, a 5-HT_{2A}, D₂, 5-HT₇ receptor antagonist with 5-HT_{1A} partial agonist properties, amisulpride, which is D₂/D₃/5-HT₇ antagonist with antipsychotic and antidepressant properties, the D₂/D₃ antagonist, sulpiride, the 5-HT_{2A} inverse agonist, pimavanserin, and the mGluR2/3 agonist, LY379268, to reverse PCP-induced deficits in NOR. We also tested the hypothesis that the 5-HT₇ agonist, AS19, would block the effect of lurasidone and amisulpride to reverse the PCP-induced deficit.

Methods: Female Long-Evans rats received vehicle or PCP (2 mg/kg) for 7 days, followed by a 7-day washout period (n = 6-9 per group). On the test day, the rats were treated with SB269970 (0.1-1 mg/kg), lurasidone (0.03-0.1 mg/kg), amisulpride (1-10 mg/kg), sulpiride

(20 mg/kg), pimavanserin (3 mg/kg), LY379268 (1 mg/kg), AS19 (5-10 mg/kg) or selected combinations of these compounds 30 min prior to NOR testing. The procedure has been described elsewhere (Snigdha et al., 2010).

Results: SB269970, the 5-HT₇ antagonist, alone, dose-dependently improved the PCP-induced deficit in NOR. Lurasidone and amisulpride improved the deficit in NOR, whereas sulpiride only did so partially. Furthermore, sub-effective doses of lurasidone (0.03 mg/kg) or sulpiride in combination with a sub-effective dose of SB269970 also reversed the NOR deficit. However, sub-effective dose of SB269970 in combination with pimavanserin or LY379268 did not improve the deficit in NOR. AS19, a selective 5-HT₇ agonist, blocked the ameliorating effect of lurasidone and amisulpride on the PCP-induced deficit in NOR, whereas AS19 did not affect the novel object preference of vehicle-treated rats.

Discussion: These results provide evidence for the importance of more than 5-HT_{2A}, D₂ and 5-HT_{1A} receptor actions to the ability of atypical APDs to improve cognition in schizophrenia. They indicate that 5-HT₇ antagonism has a procognitive effect on the PCP-induced impairment in NOR, and that 5-HT₇ antagonism contributes to the ability of lurasidone and amisulpride to ameliorate the PCP-induced NOR deficit. These results are inconsistent with previous studies which suggested 5-HT₇ agonism may promote memory, at least in the hypoglutamatergic rat brain. Moreover, these results suggest that 5-HT_{2A} antagonism and mGluR2/3 agonism are not necessary for 5-HT₇ blockade to ameliorate the PCP-induced deficit in NOR. Thus, selective 5-HT₇ antagonism may represent a novel approach for improvement of cognitive impairment in schizophrenia, if indeed, there is a hypoglutamatergic deficit in schizophrenia. Further, the combination of a 5-HT₇ antagonist, and at least some atypical APDs, should be tested as a means to enhance the efficacy of atypical APDs to improve cognition in schizophrenia. Our results add to the evidence that 5-HT₇ antagonism is a critical component to the efficacy of amisulpride (Abbas et al. 2009), which should no longer be considered a selective D₂/D₃ antagonist (Möller, 2003) or support for the so-called fast-off hypothesis (Kapur and Seeman, 2000). Further, the view that the ability of amisulpride to improve cognition in schizophrenia is independent of serotonin (Wagner et al. 2005) is unlikely to be correct. Determination of whether the results reported here translate into clinically effective treatments of the cognitive impairment in schizophrenia will provide critical tests of the validity of the hypoglutamatergic hypothesis of cognitive impairment in schizophrenia.

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213. Identification of TRPV1 Allosteric Modulators using Chemical Library High-Throughput Screening Approaches for Discovery of Non-Opioid Analgesics

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Background: Pain is one of the main reasons patients seek medical care, yet adequate pharmacological control of pain is frequently inadequate and plagued with undesirable side effects. A deeper understanding of the molecular basis for sensing noxious stimuli and tissue damage, as well as the neural circuits in which the molecules are embedded, and pathophysiological environment in which they function, is necessary for further progress. The transient receptor potential ion channel, family V, number 1 (TRPV1), is expressed in A-delta and C-fiber primary afferent nociceptors. TRPV1 is activated by capsaicin, low pH, and noxious heat and integrates a variety of

inflammatory mediators; as such it is a potential target for analgesic drug development^{2,3,4}. While this ion channel is undeniably well placed in the pain pathway, and the idea of blocking pain signals before they enter the CNS is an excellent concept, recent clinical trials with orthosteric capsaicin antagonists have encountered impediments because of their tendency to cause hyperthermia and the global lack of sensitivity to noxious heat yielding a potential for burns. We propose a new approach involving allosteric modulation as a strategy to impose an activity-dependent constraint, within the context of inflammation and tissue damage, in a way that an orthosteric vanilloid antagonist cannot achieve. The main goal of this research is to identify new, potent allosteric modulators of TRPV1 that can be used to investigate TRPV1 function and possibly act as non-opioid analgesics.

Methods: TRPV1 is a Na⁺/Ca²⁺ cation channel. The initial characterization of a library of dihydropyridine derivatives was performed using a combination of ⁴⁵Ca²⁺ uptake, calcium imaging and electrophysiology performed on either NIH3T3 or HEK293 cells stably transfected with TRPV1 or on primary cultures of rat or mouse dorsal root ganglion neurons. High throughput screening of the Molecular Libraries Probe Production Centers Network small molecule library was performed on the ectopically expressing TRPV1 cells with a no-wash calcium fluorescence assay in 1536 well format using a Hammamatsu FDSS dynamic fluorescent plate reader. The assay was conducted by a baseline read of each well followed by addition of test drug and then addition of an EC₂₀ concentration of capsaicin. An increase in the EC₂₀ signal was taken as evidence for PAM activity.

Results: Screening of the dihydropyridines revealed the presence of several PAMs. One of these, MRS-1477 was characterized further. This compound had little or no intrinsic agonist activity when applied to ectopically expressing cells or DRG neurons. However, enhancement of capsaicin and other vanilloid agonists, such as NADA, olvanil or resiniferatoxin was observed with three different endpoints: ⁴⁵Ca²⁺ uptake, free intracellular calcium concentration, transmembrane current recorded by whole cell patch clamp. Both efficacy and sensitivity were enhanced. Activation by low pH was also enhanced. The activation by acidic conditions was not blocked by capsaicin orthosteric antagonists; an observation consistent with the idea that the MRS-1477 allosteric site modulates both capsaicin and pH modes of ion channel activation independently of each other. In an effort to obtain more potent TRPV1 PAMs we screened an extensive small chemical library (~300,000 compounds). This screen yielded a variety of chemical entities that induced an increase in capsaicin-stimulated free intracellular Ca²⁺ concentration which are being characterized further with calcium and electrophysiological methods.

Discussion: The search for new mechanism-based analgesics capable of treating severe to moderate pain and thereby replace or augment opioid analgesics has proven to be exceedingly difficult. The present studies suggest a new pharmacological approach to manipulating the TRPV1 ion channel that involves allosteric modulation. The action of the lead compound appears to influence multiple modes of channel activation from, potentially, a single allosteric site. Whether the compounds identified in the HTS screen act at the same site or additional sites is being determined. The use of allosteric sites in TRPV1 for therapeutic purposes may represent a method to modulate inflammatory pain conditions which are typified by both low pH and cellular damage that can release endovanilloid compounds. We hypothesize that a TRPV1 PAM may tip the balance of calcium regulation in active nerve ending to produce a local calcium inactivation and a long duration, non-opioid analgesic action.

1. Gavva N 2008
2. Krause J et al 2005
3. Gunthorpe & Szallasi 2008
4. Patapoutian A et al 2009
5. Roh EJ et al 2008

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214. VU0152100, A Selective Positive Allosteric Modulator of M4 Muscarinic Acetylcholine Receptors Produces Efficacy in Preclinical Models of Antipsychotic-Like Activity, Negative Symptoms and Enhancement of Cognition in Rodents

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Background: Recent studies indicate that selective activators of specific subtypes of muscarinic acetylcholine receptors (mAChRs) may provide a novel approach for the treatment of psychotic symptoms associated with many psychiatric and neurologic disorders, including schizophrenia and Alzheimer's disease (AD). For example, the M1/M4-preferring mAChR agonist xanomeline produces robust decreases in psychotic symptoms, behavioral disturbances, and some of the cognitive impairments in schizophrenic and AD patients. At present, the relative contributions of M1 and M4 mAChRs to the clinical effects of xanomeline or its effects in associated animal models remain unknown. Recent findings using postmortem brain tissue from schizophrenia patients and M4 knockout (KO) mice suggest that selective activation of M4 does contribute to the effects of xanomeline and that selective activators of M4 may have exciting potential as novel antipsychotic and cognitive enhancing agents. Unfortunately, previous attempts to develop highly selective agonists of M4 have failed due to the high sequence conservation of the orthosteric acetylcholine (ACh) binding site of the mAChRs. Recently, we reported the development of VU0152100, a highly selective and systemically active positive allosteric modulator (PAM) for the M4 muscarinic receptor. We have now used VU0152100 to further assess the effects of selective activation of M4 in rodent models that predict antipsychotic or cognition-enhancing effects.

Methods: All *in vivo* microdialysis and behavioral studies were conducted using male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 250 to 300 g. Subjects were housed in pairs in a large colony room under a 12-h light/12-h dark cycle (lights on at 6:00 AM) with food and water provided ad libitum. Test sessions were performed between 6:00 AM and 6:00 PM. Dose groups consisted of 6-10 rats per dose group. All doses of VU0152100 refer to the salt form and were injected in a 1.0 ml/kg volume. The compound was dissolved in 10% Tween 80 and double deionized water with the pH adjusted to approximately 7.0 using 1 N NaOH. For the *in vivo* microdialysis and behavioral studies, rats were pretreated with vehicle or VU0152100 i.p. for 30 min prior to vehicle or a dose of apomorphine, amphetamine or phencyclidine, then evaluated for changes in locomotor activity, prepulse inhibition (PPI), social interaction, and contextual-conditioned fear responses or alterations in extracellular levels of dopamine in the medial prefrontal cortex, nucleus accumbens and dorsal striatum. Data were analyzed by a one-way ANOVA with comparison with the vehicle + amphetamine or vehicle + PCP control group using Dunnett's test. Calculations were performed using JMP version 5.1.2 (SAS Institute Inc., Cary, NC) statistical software.

Results: Here we report the characterization of VU0152100 in several preclinical models predictive of antipsychotic-like activity, negative symptoms of schizophrenia and enhancement of learning and memory tasks. In particular, VU0152100 produced robust dose-dependent reversal of amphetamine-induced disruption of prepulse inhibition of the acoustic startle reflex with effects similar in magnitude to the effects observed after administration with the M1/M4-preferring mAChR agonist xanomeline or other clinical available antipsychotics. Using *in vivo* microdialysis techniques, VU0152100 reversed amphetamine-induced increases in extracellular dopamine levels in the nucleus accumbens within the same dose range used to block induction of amphetamine-induced hyperlocomotion. In addition, VU0152100 produced robust reversals of PCP-induced hyperlocomotion and disruption of PPI and social interaction, a preclinical model of negative symptoms observed in schizophrenia. We also evaluated the effects of selective activation of M4 by VU0152100 on amphetamine-

and PCP-induced disruptions of a preclinical model of hippocampal working memory, specifically context-mediated conditioned fear response. Interestingly, VU0152100 produced dose-dependent reversal of both amphetamine- and PCP-induced disruptions of context-mediated conditioned fear responses.

Discussion: The present findings represent a critical breakthrough in this field and provide further support for the concept that highly selective M4 activators, such as M4 PAMs, have potential for development as novel antipsychotic and cognitive enhancing agents.

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215. Neural Substrates of Methamphetamine Place Reinforcement Learning: The Role of the Hippocampus-VTA Loop

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Background: Addiction is a major health problem in the United States. The reinforcing effects of addictive drugs involve the midbrain *ventral tegmental area* (VTA) which is the primary source of dopamine (DA) to the *nucleus accumbens* (NAc) and the hippocampus (Hippo). These three brain regions make a behaviorally functional connection called the Hippo-VTA loop. There are two major neural pathways within this loop: the bottom-up link; VTA projections directly into cortical and subcortical areas and a top-down link; indirect Hippo projections via the NAc and the *ventral pallidum* into the VTA. Therefore, it is likely that the Hippo (specifically the ventral hippocampus, vHippo) involves learning processes subserving addiction.

Methods: We addressed the role in conditioned place preference (CPP) learning of these two pathways by sequentially conditioning each of the three nuclei. Post baseline, rats underwent an experiment module consisting of two conditioning trials (15 min/day) followed by an immediate testing in each day (day 1, test 1; and day 2, test 2), then two post conditioning CPP tests on day 3 (test-3) and day 10 (test-4). The module was repeated three times for each nucleus. The order of conditioning was VTA, then vHippo, and finally NAc (for bottom-up) or vHippo, then VTA, and finally NAc (for top-down).

Results: The results showed that METH, but not ACSF, produced positive place reinforcement learning post conditioning in each brain area in the bottom-up order, however, in the top-down order METH, but not ACSF, produced negative (aversive) place reinforcement learning. In addition, METH place aversion was antagonized by co-administration of the NMDA antagonist MK801 suggesting the aversion learning was an NMDA receptor activation dependent process.

Discussion: We conclude that the hippocampus is critically important structure in the reward circuit. We suggest that development of pharmacotherapy for the control of addiction target the vHippo-VTA top-down connection [Note: Some of the data from this project were published as abstracts in Society for Neuroscience 2009 and 2010

Conferences (Keleta et al., SfN 2009; Keleta et al., SfN 2010). The project was supported by the Ewing Halsell Distinguished Chair and the APA T32 MH18882].

Disclosure: Y. Keleta: None. J. Martinez: None.

216. Effects of the Positive GABA-B Receptor Modulators, CGP7930 and rac-BHFF, in Animals Trained to Discriminate Baclofen or Gamma-hydroxybutyrate (GHB) from Saline

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Background: GABA-B receptors can be directly activated by agonists, but their activity can also be modulated indirectly. Because GABA-B receptors are implicated in various psychiatric disorders, modulation of these receptors offers the possibility to develop new treatments. CGP7930 and rac-BHFF are positive modulators of GABA-B receptors that can enhance *in vivo* effects of the GABA-B receptor agonist baclofen, as assessed by loss of righting in mice (Carai et al., EJP 504:213, 2004; Malherbe et al., BJP 154:797, 2008; Koek et al., JPET (in press), 2010). The present study examined the generality of these findings by studying the ability of the positive modulators to enhance discriminative stimulus effects of baclofen and of GHB (which has GABA-B receptor agonist properties) in pigeons.

Methods: Discriminative stimulus effects of drugs in pigeons were examined using methods detailed elsewhere (e.g., Koek et al., JPET 317:409, 2006). One group of pigeons ($n=8$) was trained to discriminate 7.5 mg/kg baclofen from saline, and another group ($n=8$) was trained to discriminate 178 mg/kg GHB from saline. All drugs were administered *i.m.*, except the GABA-B receptor modulators, which were given *p.o.*

Results: In pigeons trained to discriminate baclofen from saline, baclofen and GHB dose-dependently increased responding on the baclofen-appropriate key to a maximum of 93 and 100%, respectively. GHB was about 18-fold less potent than baclofen. The GABA-B receptor antagonist CGP35348 (320 mg/kg) blocked the discriminative stimulus effects of baclofen and GHB. When given alone, CGP7930 and rac-BHFF substituted partially for baclofen (maximum percentage responding on the baclofen-appropriate key: 41% after 178 mg/kg CGP7930, and 74% after 320 mg/kg rac-BHFF). At the highest dose that produced less than 25% responding on the baclofen-appropriate key when given alone (i.e., 100 mg/kg CGP7930; 32 mg/kg rac-BHFF), both modulators shifted the baclofen dose-response curve about 6-fold to the left. In contrast, the dose-response curve for GHB in pigeons discriminating baclofen was shifted less than 3-fold to the left by CGP7930 and rac-BHFF. In pigeons trained to discriminate GHB from saline, baclofen, CGP7930, and rac-BHFF produced little responding on the GHB-appropriate key: 38% after 10 mg/kg baclofen, 15% after 320 mg/kg CGP7930, and 49% after 178 mg/kg rac-BHFF). At the highest dose that produced less than 25% responding on the GHB-appropriate key when given alone (i.e., 320 mg/kg CGP7930, 56 mg/kg rac-BHFF), both modulators shifted the baclofen dose-response curve about 2-fold to the left. In contrast, both modulators shifted the GHB dose-response curve less than 2-fold to the left.

Discussion: Together with previous findings, the current results show that CGP7930 and rac-BHFF enhanced not only the loss of righting induced by baclofen, but also the discriminative stimulus effects of baclofen. CGP7930 appeared to be 3- to 6-fold less potent than rac-BHFF to enhance the discriminative stimulus effects of baclofen. These results, together with the finding that CGP7930 and rac-BHFF substituted at most partially for baclofen, demonstrate the effectiveness of CGP7930 and rac-BHFF *in vivo*, and are consistent with their *in vitro* characterization as positive GABA-B receptor modulators. Previously, we found that the GABA-B receptor antagonist CGP35348 more potently antagonizes behavioral effects of baclofen than of GHB (e.g., Koek et al., JPET June 29, 2009). Here, the positive GABA-B receptor modulators rac-BHFF and CGP7930 more effectively

enhanced the discriminative stimulus effects of baclofen than those of GHB. Together, these findings are further evidence that the GABA-B receptor mechanisms mediating the effects of GHB and prototypical GABA-B receptor agonists are not identical. A better understanding of the similarities and differences between these mechanisms, and their involvement in the therapeutic effects of GHB and baclofen, could lead to more effective medications. Supported by DA15692 (WK), DA17918 (CPF).

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217. Serotonin and Behavioral Disinhibition in Adolescent Rats

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Background: Disinhibited, risk taking behavior peaks during adolescence. Immature neural circuitry that regulates motivated behavior is thought to contribute to this risk taking behavior. Deficits in serotonergic function are associated with disinhibition, impulsive aggression, and risk taking in adults, which suggest that immaturity of serotonergically-mediated behavioral inhibition might contribute to adolescent risk taking. The present study tested this hypothesis.

Methods: We used pharmacologic and neurochemical strategies to assess the role of serotonin in tests of anxiety/behavioral inhibition in adolescent (PN 28) and adult (PN 70) rats. Acute administration of serotonergic agonists typically increases anxiety-like behavior, which has been interpreted as eliciting behavioral inhibition in a threatening situation. To evaluate this response in adolescents and adults, we administered fenfluramine (2 mg/kg), MDMA (5 mg/kg), meta-chlorophenylpiperazine (mCPP, 1 mg/kg), fluoxetine (10 mg/kg), diazepam (1 mg/kg) or vehicle to rats prior to performance of the light/dark (LD), test for anxiety to probe age differences in serotonergic function. Fenfluramine and diazepam were also tested in the elevated plus maze (EPM). Tissue content of 5 HT and 5 HIAA in frontal cortex, amygdala, and hippocampus were analyzed by HPLC. Finally, serotonin release from the frontal cortex at baseline and after fenfluramine was determined by microdialysis followed by HPLC to evaluate frontal cortex serotonin function. Behavioral results were analyzed by 2 way ANOVA (age x treatment) and microdialysis results were analyzed by repeated measures 3 way ANOVA (age x treatment x time).

Results: Adolescents performed similarly to adults in both tasks, except that they consistently emerged faster into the light than adults. Frontal cortex serotonin content correlated negatively with time in light in adult rats ($r^2 = -.63$, $p < .003$), but this correlation was not significant in adolescents ($r^2 = -.21$, ns), supporting a role for serotonin in this behavior. Diazepam was equally anxiolytic at both ages, and the direct agonist mCPP was equally anxiogenic in both age groups. However, responses to indirectly-acting serotonergic agents differed significantly by age: fenfluramine, fluoxetine and MDMA were significantly more anxiogenic in adults than in adolescents in the LD test. In the EPM, diazepam was similarly anxiolytic in adults and adolescents, but fenfluramine was significantly more anxiogenic in adults than in adolescents. Both tissue serotonin content and fenfluramine-induced increase in extracellular serotonin in frontal cortex were significantly less in adolescent than adult rats.

Discussion: The behavioral findings showed that serotonergic modulation of behavior in anxiety models (which entails risk assessment and behavioral inhibition in response to threat) is less in adolescents than adults. The difference between drugs that act presynaptically and the direct receptor agonist suggests that incomplete afferent input by serotonergic fibers mediates this difference. The latter conclusion is also supported by the microdialysis findings. Overall, these results suggest that serotonergic innervation of frontal cortex circuits that contribute to behavioral inhibition and suppression of impulsivity are relatively immature in adolescent compared to adult rats. This immaturity contributes to less behavioral inhibition in

conditions of threat, and could contribute to adolescent risk taking. Supported by DA 019114.

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218. Lack of Ketamine-like Discriminative Effects of GLYX-13: A novel NMDA Receptor Glycine Site Functional Partial Agonist with Antidepressant-like Preclinical Effects

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Background: Clinical studies with the NMDA receptor (NMDAR) antagonists CP-101,606 and ketamine have demonstrated significant reductions in depression scores in patients with treatment-resistant depression. Recently, ketamine was also reported to produce a robust antidepressant effect in patients with treatment-resistant bipolar disorder. The efficacy in these studies was profound with fast onset and long duration of effect. These findings have established the NMDAR as a novel target of high interest in the treatment of depression. GLYX-13 is a glycine-site functional partial agonist (GFPa) at the NMDAR. It has been shown to have a unique pharmacological profile compared to other NMDAR modulators to enhance hippocampal-dependent learning in young adult and learning-impaired aged rats, to simultaneously elevate long-term potentiation (LTP) while reducing long-term depression (LTD), and to alleviate formalin-induced pain. Previous studies showed that GLYX-13 had antidepressant-like activity in the rat Porsolt swim test by the i.v. and s.c. routes of administration similar to ketamine, but did not reduce prepulse inhibition or conditioned place preference as did ketamine. Likewise, in locomotor activity studies, GLYX-13 did not have any stimulatory or sedative-like effects over an i.v. dose range from 5 to 500 mg/kg. Thus, GLYX-13 appeared to have the beneficial effects of ketamine without ketamine's deleterious effects in these studies of preclinical pharmacology. In the current study, GLYX-13 was examined over an extensive s.c. dose range in rats trained to discriminate injections of ketamine from saline to determine if GLYX-13 had any potential for production of ketamine-like discriminative effects.

Methods: Adult male Sprague-Dawley (SD) rats (Charles River) were trained to discriminate i.p. injections of 10 mg/kg of ketamine HCl from injections of saline administered 10 minutes before the training sessions. All animals were well experienced in this procedure and had been previously used in the study of other NMDAR modulators. The rats responded under a FR-10 schedule of food reinforcement on one lever when they had been injected with the training dose of ketamine and on a different lever when they had been injected with saline. Animals were trained daily and the correct lever alternated under a double alternation schedule. When performance was stable under the training conditions, test sessions were conducted on Tuesdays and Fridays. On test sessions, the animals were administered varying doses of ketamine by either the i.p. or s.c. route of administration or varying doses of GLYX-13 by the s.c. route 30 minutes before the test session began. During test sessions, completion of the FR requirement on either lever resulted in food presentation.

Results: IP ketamine dose-dependently substituted for the ketamine training dose with doses of 10, 17, & 30 mg/kg producing full substitution. The doses of 17 & 30 mg/kg also reduced rates of responding during those test sessions. When administered s.c., the ketamine dose effect curve for both substitution and rate suppression were shifted to the left. In contrast, GLYX-13 over the s.c. dose range of 3, 10, 30, 56, 100, and 170 mg/kg administered 30 minutes before testing did not produce any ketamine-like discrimination or any suppression of response rates. The s.c. doses of 10 & 30 mg/kg of GLYX-13 exhibit clear antidepressant-like effects in the rat Porsolt swim test.

Discussion: The present data indicate that GLYX-13 does not have ketamine-like discriminative effects or behavioral suppressant effects over a dose range that far exceeds the "therapeutic" dose range in the

Porsolt test. This complements the previous data from preclinical studies noting no increase or decrease in locomotor activity over an extensive dose range and no conditioned place preference at a "therapeutic dose", in contrast to ketamine. The compilation of all of these results suggest that GLYX-13 may have the antidepressant effects of ketamine when tested in humans without the subjective and sedative effects of ketamine that severely limit its use for treating depression. A phase 1 clinical trial in normal human volunteers has been completed with i.v. doses of GLYX-13, and there was no sign of any sedative or ketamine-like subjective effects with plasma exposures (C_{max} and AUC) that exceeded the "therapeutic" range in the rodent preclinical pharmacology. Thus, these human data supports the lack of ketamine-like discriminative effects seen in this study. GLYX-13 has an open IND, is being developed by Naurex, Inc., and is now entering Phase II clinical trials for the treatment of depression.

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219. Neuropeptide S Alters Anxiety but not Depression-like Behaviors in the Flinders Sensitive Line Rats, a Genetic Animal Model

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Background: Neuropeptide S (NPS) and its receptor (NPSR) have been implicated in the mediation of anxiolytic-like behavior in rodents. However, little knowledge is available to what extent the NPS system is involved in depression-related behaviors. The aim of the present work was to characterize the effects of centrally administered NPS on depression- and anxiety-related behaviors, using a well validated animal model of depression, the Flinders Sensitive Line (FSL) rats and their controls the Flinders Resistant Line (FRL).

Methods: Male and female were tested. Seven days following insertion of cannula, 0.25 or 1.0 nmol NPS, or vehicle/5 µl were infused into the lateral ventricle. 45 min after NPS infusion animals were tested on elevated plus maze (EPM). Five days later the animals were subjected to the two-day forced swim test (FST); NPS or vehicle were injected 45 min before the second day FST. In selected animals effect of NPS on home cage activity was explored. Finally, brains from separate groups of naïve animals were harvested; hippocampi, amygdalae and PVN punched out, and mRNA transcripts measured with the real-time quantitative polymerase chain reaction (rt-qPCR).

Results: The most salient findings were: (1) NPS increased in a dose-dependent fashion the percent time spent in the open arms and the number of full entries in the FSL rat ($p < 0.01$), while the effects in the FRL were only marginal; (2) In contrast to altered behavior in the EPM, behavior in the FST was not affected by NPS; (3) In the home cage, NPS increased locomotion in a dose dependent fashion in the FSL; (4) rt-qPCR showed that NPSR expression was lower in amygdalae in the FSL; no other region or strain differences were found.

Discussion: Baseline depression-like behavior was markedly higher in the FSL compared to FRL rats while no difference in the anxiety-like behavior was observed. These findings confirm the utility of the FSL as a model of depression useful in exploration of neurobiological correlates both of depression and those discriminating between depression and anxiety endophenotypes. NPS had marked anxiolytic effects but did not modify the depression-like behavior. These results clearly separate effects of NPS from those of the classical antidepressants (tricyclics and SSRIs) which are also anxiolytic, and indicate that NPS agonists could be useful as selective anxiolytic drugs.

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220. Escitalopram Enhances the Risperidone-induced Antipsychotic-like Effect, Cortical Dopamine Output and NMDA Receptor-mediated Transmission. Comparison with R-citalopram and Citalopram

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Background: We have previously, in the rat, examined different pharmacological means to improve the efficacy of antipsychotic drugs by adjunct treatment with drugs that enhance noradrenergic transmission, i.e. the α_2 antagonist idazoxan, the antidepressant noradrenalin reuptake inhibitor reboxetine and the tetracyclic antidepressant drug mianserin. Here we continued to study adjunct treatment with drugs that enhance serotonergic transmission. Among the SSRIs, including citalopram, escitalopram (the S-enantiomer of citalopram) has shown advantageous efficacy in the treatment of major depressive disorder. In addition, whereas citalopram may impair cognition, escitalopram may even improve cognitive dysfunction. We have recently shown that escitalopram, but not R-citalopram (the R-enantiomer) or citalopram, increases firing rate and burst firing of dopamine neurons in the ventral tegmental area, potentiates cortical NMDA receptor-mediated transmission and enhances recognition memory, which may help explain its advantageous clinical profile. We have now studied the effects of adjunct treatment with escitalopram, R-citalopram or citalopram when added to the second generation antipsychotic drug (SGA) risperidone.

Methods: We used conditioned avoidance response to assess antipsychotic efficacy, the catalepsy test to examine extrapyramidal side effect liability, *in vivo* microdialysis in freely moving animals to measure dopamine efflux in the medial prefrontal cortex and the nucleus accumbens (NAc) and *in vitro* intracellular electrophysiological recordings in pyramidal cells to examine the effect on cortical NMDA receptor-mediated transmission.

Results: Addition of escitalopram (5 mg/kg) or citalopram (10 mg/kg) to risperidone (0.4 mg/kg) significantly enhanced the antipsychotic-like effect. Escitalopram, citalopram and R-citalopram (5 mg/kg) seemed to enhance the antipsychotic-like effect also of a low dose of risperidone (0.25 mg/kg), however, it did not reach statistical significance. Catalepsy measurement did not reveal any significant effect. Given alone, only escitalopram was able to increase cortical dopamine outflow. Addition of escitalopram to a low dose of risperidone (0.25 mg/kg) resulted in a large increase in prefrontal dopamine output but, surprisingly, also R-citalopram generated a small but significant dopamine increase. Escitalopram, R-citalopram and citalopram all enhanced the risperidone-induced dopamine outflow in the NAc. However the largest effect was obtained by citalopram and not escitalopram. Given alone, only escitalopram (5 nM) significantly enhanced cortical NMDA receptor-mediated transmission. Yet, both escitalopram (5 nM) and R-citalopram (5 nM), but not citalopram (10 nM), potentiated the effects of a sub-maximal concentration of risperidone (10 nM).

Discussion: We here show that even if all three drugs, escitalopram, citalopram and R-citalopram, to some extent may enhance the antipsychotic-like effect of risperidone, escitalopram is the most efficacious. In addition, escitalopram, to a much greater extent than both R-citalopram and citalopram, was able to enhance risperidone-induced cortical dopamine output as well as cortical NMDA receptor-mediated transmission. Previous studies propose that a marked enhancement of cortical dopamine outflow *per se* is sufficient to produce an enhanced effect of antipsychotic drugs. Moreover, cortical dopamine may regulate and functionally inhibit subcortical dopamine transmission, and enhancement of cortical NMDA receptor-mediated transmission has been shown to be dopamine-dependent. The preferential enhancement of cortical dopamine and NMDA receptor-mediated transmission by combined treatment with escitalopram and risperidone both suggest beneficial effects also on cognitive function-

ing, e.g. working memory. Previously, R-citalopram has been considered to be therapeutically inactive and, in fact, to antagonize the effects of escitalopram. Yet, whereas R-citalopram given alone was without effect, it was still able to significantly increase the effect of risperidone on both cortical dopamine output *in vivo* as well as NMDA receptor-mediated transmission *in vitro*. Our work suggests that adjunct treatment with escitalopram may enhance the effect of SGAs, such as risperidone, on positive, negative as well as cognitive and depressive symptoms in schizophrenia, and tentatively also in bipolar disorder, notably bipolar depression. In contrast to previous assumptions R-citalopram may under certain conditions exert significant psychopharmacological effects in the brain.

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221. Bupropion Exerts Cannabinoid Antagonist Activity in Rhesus Monkeys

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Background: Bupropion is an antidepressant and aid to cigarette-smoking cessation. In clinical studies examining bupropion as a treatment for marijuana dependence, bupropion worsened marijuana withdrawal symptoms (Haney et al., *Psychopharmacology* 155:171, 2001) and did not modify abstinence relative to placebo (Carpenter et al., *Am J Addict* 18:53, 2009). To further examine interactions between bupropion and cannabinoids, bupropion and pharmacologically related drugs were studied in two drug discrimination assays sensitive to acute cannabinoid action and cannabinoid withdrawal in rhesus monkeys.

Methods: Discriminative stimulus effects of cannabinoids were examined with methods reported previously (McMahon, *J Pharmacol Exp Ther*, 319:1211, 2006; Stewart and McMahon, *J Pharmacol Exp Ther*, 334:347, 2010). One group of rhesus monkeys (n=5) discriminated the cannabinoid agonist Δ^9 -tetrahydrocannabinol (Δ^9 -THC; 0.1 mg/kg i.v.) from vehicle; another group (n=5) discriminated the cannabinoid antagonist rimonabant (1 mg/kg i.v.) from vehicle while receiving chronic Δ^9 -THC (2 mg/kg/day s.c.) treatment (i.e., rimonabant-induced cannabinoid withdrawal). Bupropion and pharmacologically related drugs included for study (i.e., the indirect-acting catecholamine agonists cocaine and amphetamine and the nicotine antagonist mecamylamine) were administered s.c.

Results: In the monkeys discriminating Δ^9 -THC, the training drug (i.e., Δ^9 -THC) dose-dependently increased the percentage of Δ^9 -THC lever responses, e.g., to 94% at the training dose (0.1 mg/kg). In contrast, rimonabant (3.2 mg/kg), bupropion (10 mg/kg), amphetamine (0.32 mg/kg), cocaine (1 mg/kg), and mecamylamine (3.2 mg/kg) produced no more than 3% responses on the Δ^9 -THC lever. When combined with Δ^9 -THC, rimonabant (1 mg/kg) and bupropion (10 mg/kg) antagonized the Δ^9 -THC discriminative stimulus, evidenced by 11- and 4-fold rightward shifts in the Δ^9 -THC dose-response curve. Amphetamine (0.32 mg/kg) and cocaine (1 mg/kg) also shifted the Δ^9 -THC dose-response curve rightward, although to a lesser magnitude than that obtained with bupropion. In contrast, mecamylamine (3.2 mg/kg) did not modify the Δ^9 -THC discriminative stimulus. In Δ^9 -THC treated monkeys discriminating rimonabant, the training drug (i.e., rimonabant) dose-dependently increased the percentage of rimonabant-lever responses, e.g., to 98% at the training dose (1 mg/kg). The percentage of responses on the rimonabant lever was 32% after bupropion (3.2 mg/kg), 25% after amphetamine (0.32 mg/kg), and 46% after cocaine (1 mg/kg).

Discussion: These results demonstrate that bupropion, amphetamine, and cocaine exert cannabinoid antagonist activity in primates that is likely functional inasmuch as these catecholamine agonists do not bind to cannabinoid receptors. One implication of these findings is that bupropion and other catecholamine agonists produce, at least in part, a cannabinoid withdrawal syndrome in cannabinoid-dependent animals. Given that rimonabant, like bupropion, promotes cigarette-smoking cessation, another implication of these results is that bupropion promotes smoking cessation, in part, by antagonizing endogenous cannabinoid neurotransmitters.

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222. Mitochondrial Morphology - A Sensitive Marker for the Mitochondria Targeted Alzheimer Drug Dimebon

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Background: Mitochondrial dysfunction plays a key role in most neurodegenerative disorders including Alzheimer's disease (AD). AD animal and cell models indicate that typical histopathological alterations but also risk actors like aging are associated with functional deficits of respiratory chain complexes. Consecutively, mitochondrial dysfunction is induced, contributing to synaptic dysfunction and finally neurodegeneration. In view of this critical role of mitochondrial dysfunction for AD, mitochondrial protection and/or improvement of mitochondrial function has become an important strategy for new drugs to treat AD. A recent example is the old Russian drug Dimebon (latrepirdine), which has shown in a phase II trial in AD patients substantial clinical benefit. Originally investigated as an antihistaminic drug, Dimebon has been suggested to work by improving mitochondrial function.

Methods: Accordingly, we investigated the beneficial effects of Dimebon in several cell systems (PC12, HEK-293) where mitochondrial dysfunction was induced by the complex inhibitor rotenone or by beta-amyloid peptide. We looked for effects on the mitochondrial membrane potential (MMP), ATP production, and on mitochondrial morphology.

Results: When cells were incubated for 6 h with different Dimebon concentrations 1 h before or 1 h after challenging the cells with 25 micromolar rotenone, Dimebon significantly reduced mitochondrial dysfunction by increasing MMP and ATP levels relatively to the cells treated with rotenone alone. Using confocal laser scanning microscopy, a high density of mito-CMX-ROS labelled mitochondria with long-tubular shape were observed in the HEK-293 cells. Mitochondrial morphology was drastically changed by a similar rotenone treatment. Mitochondria were fragmented, showed punctuate morphology, and accumulated around the nucleus. Pre-incubation of the cells with Dimebon (100 nmolar) highly compensated against rotenone induced mitochondrial fragmentation. Mitochondria length and tubular shape was highly preserved. Compared to the effects on MMP and ATP-levels, effects on mitochondrial morphology were much more pronounced. Hence, we conclude that changes in morphology might be more sensitive for determining mitochondrial function compared to MMP and ATP levels. Comparable mitochondrial protection was also seen in HEK cells overproducing human beta-amyloid by stable transfection with the Swedish APP double mutation.

Discussion: Our data support the assumption that Dimebon might act in neurodegenerative diseases by protecting mitochondria or by improving mitochondrial dysfunction.

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223. Locus Coeruleus: A Novel Substrate in the Regulation of Sensorimotor Gating

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Background: Despite the large number of psychiatric illnesses in which dysfunction of noradrenergic (NE) signaling may play a part, surprisingly little is known about the modulation of specific psychopathological symptoms by discrete NE brain circuits. Prepulse inhibition (PPI) provides an operational measure of sensorimotor gating, and refers to the diminution of startle responses when weak prestimuli precede the intense startling event. Animal models of deficient PPI represent a widely validated translational paradigm with which to study the information processing deficits that are commonly observed in several mental disorders. Emerging evidence indicates that NE might regulate PPI, however the anatomical substrates for this phenomenon are not known. The present studies therefore examined the regulation of PPI by the locus coeruleus (LC), the primary source of NE to forebrain.

Methods: Using microinfusion procedures that previously have been well-established to produce an activation of LC neurons (with peri-LC infusion of the cholinergic agonist bethanechol or the glutamate agonist AMPA), male Sprague-Dawley rats were examined for PPI responses following pharmacological stimulation of LC. In separate groups of rats, this LC manipulation was combined with systemic injections of either NE, dopamine (DA), or serotonin (5-HT) receptor antagonists or different types of antipsychotics to determine if these drugs would reverse LC-mediated PPI effects.

Results: An anatomically and behaviorally specific deficit in PPI was found after activation of the locus coeruleus. This effect was blocked by clonidine (an α_2 receptor agonist that shuts off LC neuronal firing after peri-LC delivery), a postsynaptic α_1 NE receptor antagonist (prazosin), and second generation antipsychotics (Zyprexa, Quetiapine), but not by drugs that selectively antagonized D₂ (the first generation antipsychotic Haloperidol) or 5-HT₂ receptors (ritanserin). **Discussion:** These results indicate a novel substrate in the regulation of sensorimotor gating, and reveal a novel functional role for the LC in pre-attentional information processing. Hence, a hyperactive LC-NE system might underlie core behavioral deficits (i.e., disrupted sensorimotor gating) in several psychiatric illnesses, and the ability to normalize LC-NE transmission could contribute to the clinical efficacy of drugs (α_2 receptor agonists, second generation antipsychotics) that are used to treat these conditions.

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224. Fish and Chips: Behavioral and Genomic Studies of Omega-3 Fatty Acids Effects in Animal Models of Bipolar Disorder and Alcoholism

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Background: Omega-3 fatty acids have been proposed as an adjuvant treatment option in psychiatric disorders. A mechanistic understanding of their effects is needed.

Methods: Here we report studies demonstrating the phenotypic normalization and gene expression effects of dietary omega-3 fatty acids, specifically docosahexaenoic acid (DHA), in a stress-reactive circadian gene (DBP) knock-out mouse model of bipolar disorder and co-morbid alcoholism, using a convergent functional genomics approach to prioritize disease-relevant genes. Additionally, to validate the novel observed effects on decreasing alcohol consumption, we also tested the effects of DHA in an independent animal model, alcohol preferring (P) rats, an established animal model of alcoholism.

Results: Our work uncovers sex differences and multiple molecular pathways for the possible effects of DHA in bipolar and related disorders, and suggests that omega-3 fatty acids may indeed become an important adjuvant treatment for stress-driven externalizing psychiatric disorders.

Discussion: Given their other health benefits and their relative lack of toxicity, teratogenicity and side-effects, they may be particularly useful in females of child-bearing age, especially during pregnancy and postpartum.

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225. Neural Correlates of Response Reversal in Pediatric Bipolar Disorder and Severe Mood Dysregulation

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Background: The question of whether youth with severe chronic irritability should be considered to have bipolar disorder (BD) is controversial. Research suggests that neuroimaging data may differentiate clinical groups with similar behavioral deficits. The syndrome of severe mood dysregulation (SMD) was defined to capture chronically irritable children whose nosologic status with respect to bipolar disorder (BD) is debated (Leibenluft et al., 2003). To determine whether SMD is a developmental subtype of BD, it is important to compare the neurobiology of SMD to that of "classic", episodic BD. While outcome and family history data differentiate SMD and BD patients, more complex findings emerge in research on information-processing functions. Recent studies have shown that similar behavioral deficits in SMD and BD can reflect disorder-specific neural perturbations (Brotman et al., 2010; Rich et al., 2007).

We compared SMD, BD, and healthy volunteers (HV) on brain activity during response reversal (RR). Both SMD and BD youth have behavioral deficits on RR tasks, as well as on other tasks assessing cognitive flexibility. RR deficits may place a subject at risk for frustration because of an inability to adapt to changing environmental contingencies. Increased frustration may lead to greater irritability, a major symptom of both SMD and BD. Although we have documented abnormal neural activation during RR in BD compared to HV, no studies have examined the neural correlates of this process in SMD.

Methods: 34 HV, 26 BD, and 22 SMD children (mean age: 14 y) completed a probabilistic RR task during a 1.5T MRI scan. A 3x2x2 ANOVA was run in AFNI that modeled diagnosis (BD, SMD, and HV) X phase (acquisition, reversal) X accuracy (correct, incorrect). All interactions and main effects were examined. We investigated task effects in four bilateral regions of interest (ROIs): caudate, cingulate gyrus, inferior frontal gyrus (IFG), and medial frontal gyrus.

Results: Diagnosis X accuracy interactions were evident in right caudate and right IFG. Because we were interested in between-group differences across event types, we decomposed these interactions by calculating difference scores (e.g., BOLD response during Incorrect trials minus BOLD response during Correct trials) and running independent t-tests on these values. In caudate, HV had a greater increase in response to incorrect vs. correct trials than did SMD or BD. In IFG, both BD and HV showed a greater increase in response to incorrect vs. correct trials than did SMD. These data indicate that neural deficits in IFG and caudate during RR differentiate BD and SMD youth from HV and each other. Post-hoc analyses suggest that comorbid ADHD may play a role in our findings.

Discussion: This study is the first to compare the neural underpinnings of RR in HV, BD, and SMD youth. Comparing all three groups allowed us to differentiate disease-unique and disease-common abnormalities amongst SMD and BD, diagnoses with overlapping

symptoms of significant mood instability and deficits on cognitive flexibility tasks. Here we found evidence that striatal dysfunction may also be common to these two diseases. On the other hand, we found evidence for differences between these groups in prefrontal cortex function, which may be disorder-specific. Overall, the results suggest that behavioral deficits observed in prior out-of-scanner testing may be associated with a failure to show the expected increase in activation in IFG (in the case of SMD) and caudate (in the case of both SMD and BD) after negative feedback. This, in turn, suggests a mechanism for the failure of youth with SMD or BD to adapt their behavior in response to negative feedback, and potentially a source of increased frustration and irritability in these patients.

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226. Abnormal Physiology and Morphology of Raphe Serotonin Neurons Early in Development and in those Lacking the Pet-1 Transcriptional Factor

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Background: The 5-hydroxytryptamine (serotonin, 5-HT) system is a primary player in development, influencing neurogenesis, synaptogenesis, dendritic growth and pruning, axonal differentiation, and cell migration. By E21 to P4, the 5-HT neurons are in place relative to structural landmarks in the brainstem. The subfields of the DR and the MR undergo modifications, i.e., dendritic growth, decrease in cell density, synaptogenesis and redistribution of cells. What is unknown is the functional development of the raphe at the cellular level. The *Pet-1* transcription factor is required for proper 5-HT neuron development, as there is disruption of the entire 5-HT system in *Pet-1* knockout (KO) mice. There is a dramatic reduction in the expression of serotonin-specific gene expression such that 5-HT precursor neurons survive leading to an anxious and aggressive phenotype. The changes in the physiology of the 5-HT precursor cells of *Pet-1* KO mice are uncharacterized. The *Pet-1*KO mice were used to test the hypothesis that the *Pet-1*KO precursor neurons would be undeveloped.

Methods: Adult (3-5 months) *Pet-1*KO, wildtype mice, and mice in which YFP was linked to the *Pet-1* transcription factor were used, in addition to YFP pups at postnatal day 4-5. Mice were decapitated, their brains dissected rapidly to generate 200 µm-thick midbrain slices. Visualized whole-cell patch clamp recordings were conducted to obtain passive and active membrane characteristics, the magnitude of the 5-HT_{1A} receptor mediated response using 5-carboxyamidotryptamine (5-CT, 100 nM) as the agonist and glutamatergic synaptic activity. Recorded neurons were identified by biocytin immunohistochemistry, the identity of the 5-HT precursor neurons was determined by staining for beta galactosidase. For morphological analysis, xyz stacks at 0.5 µm were obtained of the biocytin labeled neurons. Analysis of the morphology was conducted using Neurolucida software.

Results: Several characteristics were similar in the *Pet-1*KO and P4 neurons and significantly different from the WT and YFP neurons. The resting membrane potential was more depolarized, $P_4 = \text{Pet-1KO} < \text{WT} < \text{YFP}$, the membrane resistance greater with $P_4 > \text{Pet-1KO} > \text{WT} = \text{YFP}$. Two indices of excitability revealed that P4 and *Pet-1*KO neurons were much more excitable, i.e., the activation gap and frequency-intensity plot; activation gap (threshold minus the action potential threshold) $P_4 = \text{Pet-1KO} < \text{WT} = \text{YFP}$ and the number of action potentials generated by depolarizing current steps was significantly greater in *Pet-1*KO and P4 neurons than either WT or YFP. The 5-HT_{1A} receptor mediated response was totally absent from the P4 neurons. The majority of the *Pet-1*KO neurons also did not exhibit a response to 5-CT administration (8 out of 14) with a mean response of 3 mV. In contrast the neurons recorded from YFP and WT mice had normal responses. Preliminary analysis of the morphology of the P4 and *Pet-1*KO neurons revealed that the *Pet-1*KO neurons had

much shorter dendrites, less branches and ends than P4 and YFP neurons. The complexity of the dendritic trees was significantly decreased in *Pet-1*KO neurons. The number of dendrites and the complexity of the P4 neurons was greater than the YFP neurons.

Discussion: The cellular characteristics of the P4 and *Pet-1*KO neurons resembled neurons that were undeveloped. The resting membrane potential was depolarized and the membrane resistance very large (decreased conductance) indicating that ion channels had not developed. The increased membrane resistance, lack of the 5-HT_{1A} autoreceptor mediated hyperpolarization and the increased excitability of the neurons implies that the neurons will be more responsive to incoming synaptic activity. The morphology of the neurons of the *Pet-1*KO mice had dendrites that were undeveloped and undifferentiated. The P4 neurons in contrast had extensive dendrites that were complex, more than the YFP neurons, implicating that the dendrites undergo pruning later in development. These results regarding the neurons recorded from the *Pet-1*KO mice demonstrate the importance of the *Pet-1* transcription factor for normal neuronal development, i.e., synaptogenesis, dendritic growth and differentiation, and ion channel insertion. These characteristics may also be important in terms of the anxiety and aggressive phenotype of the *Pet-1*KO mouse.

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227. Organic Cation Transporter 3: The Culprit Undermining Therapeutic Utility of SSRIs?

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Background: Depression is a major health problem compounded by the inability of currently available medications to effectively alleviate symptoms in a large majority of patients (STAR*D reports). The increase in extracellular serotonin (5-HT) that follows administration of many commonly prescribed antidepressants, including selective 5-HT reuptake inhibitors (SSRIs), is thought to be one of the keys to triggering the cascade of events that ultimately lead to therapeutic benefit. One reason why these antidepressants may lack therapeutic efficacy is the existence of multiple other transporters in brain capable of 5-HT uptake, thereby preventing extracellular 5-HT rising sufficiently high to trigger this cascade. A growing body of work from our lab, and others, points to the organic cation transporter 3 (OCT3) as a key 5-HT transporter that may mute or prevent therapeutic benefit in depressed patients and which may be a new target for the development of novel antidepressant drugs.

Methods: We used a combination of *in vivo* electrochemistry, genetic, pharmacological and behavioral approaches to investigate the antidepressant potential of compounds targeting OCT3.

Results: One patient population for which SSRIs are particularly ineffective in treating depression is that comprising individuals with variants of the serotonin transporter (SERT) gene that confer reduced expression of this transporter. Using SERT knockout (KO, -/-) mice, which lack SERT, and SERT heterozygote (+/-) mice, which constitutively express half as many SERT as wildtype (+/+) mice and provide a model for this patient population, we found that OCT3 expression and function was increased relative to +/+ mice. Importantly, acute i.p. administration of decynium-22 (D-22), a blocker of OCT3, produced antidepressant-like effects in the tail suspension test (TST) in SERT mutant mice, a behavioral effect that was time locked to the inhibition of 5-HT clearance in hippocampus produced by D-22 (Baganz et al., 2008, PNAS). Likewise, we also found that the ability of ethanol to inhibit 5-HT clearance in hippocampus was greatest in SERT mutant mice. This effect was potentiated by concurrent administration of an SSRI or a selective norepinephrine reuptake inhibitor, but not by corticosterone, a potent blocker of OCT3, suggesting that OCT3 might be a site of action for ethanol's inhibitory effect on 5-HT uptake. Finally, to determine if repeated

OCT3 blockade might produce antidepressant-like effects in wild-type mice, we exposed mice to a regime of repeated swim (10 min/day for 14 days). This paradigm leads to robust increases in plasma corticosterone that we found persisted even when measured 24 h after the last swim. We found that, 24 h after the last swim, 5-HT clearance rate was dramatically slowed in these mice, an effect that was absent in adrenalectomized mice indicating the need for an intact hypothalamic-pituitary-adrenal (HPA) axis in order to produce this effect of repeated swim to inhibit 5-HT clearance. Consistent with slowed 5-HT uptake having antidepressant-like effects, mice exposed to repeated swim spent less time immobile in the TST.

Discussion: The key findings from these studies support the ideas that (1) OCT3 can act to keep the brake on extracellular levels of 5-HT, in individuals with a full complement of SERT but even more so when SERT expression is constitutively reduced, thereby buffering the ability of SSRIs to increase extracellular 5-HT; (2) OCT3 is a putative site of action for ethanol. While requiring further investigation this finding may provide a mechanistic basis for reports that humans carrying low expressing SERT variants are more prone to alcoholism, perhaps as a form of self-medication for depression and related disorders; and (3) OCT3 blockade (D-22, corticosterone) produces antidepressant-like effects. We and others have shown that under certain conditions and doses, corticosterone can produce antidepressant-like effects. Clearly corticosterone itself is not a viable candidate for the treatment of depression due to its many and varied actions at other receptors and clear pro-depressant effects under many conditions. However, the major implication from this growing body of work is that OCT3 is an important modulator of brain 5-HT and a promising candidate for the development of novel, OCT3 selective drugs for the treatment of depression and related disorders. Supported by MH64489 (LCD) and NARSAD (LCD and WK).

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228. Age-Related Loss Of Hypothalamic Orexin/Hypocretin Neurons

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Background: Hypothalamic orexin/hypocretin neurons influence several homeostatic functions, such as arousal and energy balance, which are impacted in aging. Orexin neurons also modulate the activity of telencephalic areas implicated in age-related cognitive decline, including the basal forebrain cholinergic system and prefrontal cortex. Age-related changes in orexin peptide expression, receptor expression and innervation of some of these regions have been described. However, there have been few systematic evaluations of the main effect of age on the number of orexin neurons, and virtually no reports on how aging might differentially impact the functionally-distinct medial and lateral banks of these cells.

Methods: Here, young (3-4 months old) and aged (26-30 months old) male Fisher 344/Brown Norway rats were euthanized and their brains processed for immunohistochemical assessment of cell numbers in both medial and lateral (relative to the fornix) sectors of the lateral hypothalamus/perifornical area.

Results: Unbiased stereological analysis revealed that young animals had an average of 3998 orexin neurons whereas old animals had an average of 2339 orexin neurons, a statistically significant decrease of greater than 40%. This decrease was of roughly equivalent magnitude in both medial and lateral sectors. To determine the specificity of the reduction in orexin neurons, we also performed stereological assessment of melanin concentrating hormone- (MCH-) expressing cells as well as the total number of lateral hypothalamic/perifornical neurons using the pan-neuronal marker, NeuN. MCH neurons were significantly decreased in aged animals, although the magnitude of reduction (29%) was not as robust as that observed with orexin labeling. There was no age-related change in the total number of NeuN-positive cells in the LH/PFA.

Discussion: Collectively, these data suggest that the orexin/hypocretin system is particularly vulnerable to age-related neurodegeneration. Loss of these neurons, or their phenotypic silencing, is likely to contribute to age-related changes in homeostasis and cognition. Supported by a University of South Carolina Magellan Scholarship (BAK) and NIH R01AG030646 (JF).

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229. Neurochemical Response Determined with ¹H-MRS to the Noradrenergic Alpha-2A Agonist Dexmedetomidine

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Background: Modulation of noradrenergic neurotransmission by agonist stimulation of NE alpha-2A receptors is a clinically effective strategy for improving attention deficits and executive function (guanfacine), pre-operative sedation (dexmedetomidine), and hypertension (clonidine). Treatment of PTSD with alpha-2A R agonists has produced mixed results. Additionally, in animal models NE alpha-2A R stimulation decreases: 1) glutamate release from primary spinal afferents thus exerting antinociceptive properties, 2) ketamine-induced dopamine release in the frontal cortex of a model of psychosis, 3) glutamate release in the stria terminalis thereby decreasing stress-induced drug seeking behaviors, and 4) insulin release from pancreatic beta cells with a resultant hyperglycemia. Given the importance of alpha-2A R activation in neuropsychiatry and pain management, we used ¹H-MRS to determine regional neurochemical profiles in rats treated acutely with the selective alpha-2A R agonist dexmedetomidine.

Methods: Thirty or 90 min after treatment with dexmedetomidine (50 µg/kg sc) or saline, male Sprague Dawley rats (~300 g) were sacrificed, brains removed, and regions of interest (1.5 mm punches) obtained from frozen 2 mm coronal slices. Intact tissue samples (~2 mg) were analyzed with magic angle spinning ¹H-MRS at 11.7T (500 MHz Bruker) and 20 neurochemicals quantified (nmol/mg) with a custom LCModel constructed from 29 known standards and non-specific lipid resonances. Goodness of fit between the LCModel and individual compounds in the chemical shift spectrum was determined with Cramer-Rao lower bounds. Statistical analysis was a 2-tailed t-test (p<0.05, saline vs treated) for each neurochemical.

Results: Within 10-15 min, dexmedetomidine treated animals lost their righting reflex as well as response to toe-pinch, consistent with the clinical use of dexmedetomidine as a pre-operative sedative. Glutamate (GLU) decreased at both time points in the VL-thalamus and accumbens (but not dorsal posterior striatum); after 90 min GLU and GABA decreased in the medial prefrontal cortex (MPF); neither GLU, glutamine (GLN), nor GABA were affected in either the rostral or caudal cingulate cortex. In the rostral cingulate cortex, dexmedetomidine treatment increased glycine, lactate (LAC), glycerophosphorylcholine (GPC), and glutathione. In fact, LAC was increased and succinate (SUC) decreased in VL-thalamus, cingulate and MPF cortices, cerebellum, and accumbens. Increased GLN/GLU in the VL-thalamus and MPF was driven by the GLU decrement.

Discussion: The results provide insight into the acute regional neurochemical response to a sedative dose of the alpha-2A agonist dexmedetomidine. Notably, the effects of dexmedetomidine on GLU and GABA were restricted to the thalamus, MPF, and accumbens; This pattern contrasts our previous observations with GABA-A receptor mediated sedation where propofol and inhaled anesthetics had significant effects on neurotransmitter content in the cingulate cortex. Disrupted GLN/GLU homeostasis in the thalamo-cortical-striatal regions may reflect an initial NE modulation of GLU release from thalamo-cortico neurons as well as a direct hyperpolarizing effect on GLU containing neurons in the MPF. Since the efficacy of

monoaminergic enhancement of executive function is U-shaped with respect to dose, it remains to be determined if the present results would similarly characterize a state of enhanced attention. NE alpha-2A R mediated inhibition of pancreatic insulin release and subsequent hyperglycemia likely influenced the LAC (seen in all brain regions) and SUC response, possibly reflecting a hyper-metabolic astrocyte response combined with drug-related neuronal quiescence. Finally, the results demonstrate the utility of ¹H-MRS to assess neurochemical responses to therapeutic agents. Support: Fund for Medical Research (Anesthesiology), Joe Young Sr Fund for Psychiatry Research (Psychiatry).

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230. Norepinephrine Transporter A457P Knockin Mouse: A Model of a Gene Variant Associated with Comorbid Cardiovascular and Psychiatric Disorders

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Background: Norepinephrine (NE) serves as a neurotransmitter in both the brain, where it mediates processes underlying arousal, attention, memory emotion, and the response to stress, and in the sympathetic nervous system. NE neurotransmission is involved in the pathogenesis of cognitive and mood disorders including attention deficit hyperactivity disorder (ADHD), major depression and anxiety as well as cardiovascular diseases. NE released at synapses in the brain and sympathetic nervous system is actively transported into terminals by the presynaptically-localized norepinephrine transporter (NET), making it a critical mediator of NE inactivation and presynaptic catecholamine homeostasis. In the heart, in particular, NET is highly effective in controlling synaptic NE and manipulations of NET have a profound effect on heart rate. NET is a target for tricyclic antidepressants, NET-selective reuptake inhibitors, and psychostimulants, all of which have served as important therapeutics for ADHD and affective disorders. Thus, disruption of NET activity may produce comorbid psychiatric and cardiovascular symptoms. We previously identified a single nucleotide polymorphism in the human NET gene, A457P, in a family with incidence of the cardiovascular disorder, orthostatic intolerance (OI), demonstrating highly elevated heart rate and plasma NE upon standing. A457P family members are also diagnosed with ADHD. *In vitro* expression studies demonstrate that A457P is a loss-of-function transporter with a dominant-negative influence on wild-type (WT) NET.

Methods: "Knockin" mice expressing NET A457P were generated. Adult, littermates of the NET A457P knockin mouse line were examined for total and plasma membrane levels of NET, and NE transport in synaptosomes from NE terminals. For transporter expression and activity studies, mice were decapitated, frontal cortex and hippocampus dissected, and synaptosome preparations were made. For NE transport assays, synaptosomes were incubated for 10 min with 150 nM [³H]NE and specific activity was defined using 10 µM desipramine. Cell-surface biotinylation assays were performed utilizing cell-impermeant biotinylating reagents to label synaptosome surface proteins. Following immunoprecipitation of biotinylated fractions with avidin beads, both total and biotinylated fractions were subjected to SDS-PAGE and Western blotting with an antibody to NET (Mab Technologies).

Results: In heterozygous A457P knockin mice, surface levels of NET were reduced to 69.4 ± 3.7 and 73.5 ± 8.6% of WT in cortex and hippocampus, respectively. Transport levels were 60.7 ± 5.4 and 63.5 ± 4.7% of WT in cortex and hippocampus, respectively. In homozygous knockin mice, transport was reduced to 13.8 ± 2.5 and 7.5 ± 2.4% of WT in cortex and hippocampus, respectively, despite surface levels of NET that were not extensively compromised.

Discussion: These data demonstrate that A457P is both trafficking- and activity-deficient in neurons *in vivo*. Studies are underway to determine the contribution of transport deficiency to the hyperadrenergia, tachycardia and cognitive symptoms observed in human carriers. Genetic disruption in genes, such as NET, with shared expression in the brain and autonomic nervous system, may explain comorbidity of psychiatric and cardiovascular disease.

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231. Resting Regional Brain Metabolism in Healthy Humans Predicts Extinction Recall and Associated Brain Activations: A Combined PET and fMRI Study

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Background: Augmented fear expression and reduced fear extinction are at the core of many anxiety disorders. Functional and structural magnetic resonance imaging studies in humans suggest that the dorsal anterior cingulate cortex (dACC) is involved in mediating fear responses, whereas the ventromedial prefrontal cortex (vmPFC) is involved in recall (retention) of fear extinction learning. However, it is unknown whether resting regional cerebral metabolism in these regions is predictive of either retention of fear extinction or associated recall-induced activation in these same brain areas.

Methods: Twenty healthy subjects underwent a ¹⁸fluorodeoxyglucose positron emission tomography (FDG-PET) scan to measure resting regional brain metabolism. Subsequently, the subjects underwent a

differential fear conditioning paradigm using a mild aversive electric shock to the fingers as the unconditioned stimulus while they were in a functional magnetic imaging (fMRI) scanner. Differently colored lights served as two reinforced CS+ and one non-reinforced CS- stimuli. Following conditioning, one of the two CS+s was extinguished, whereas the other was not. The next day, the extinguished CS+ (CS+E), the unextinguished CS+ (CS+U), and the CS- were presented during extinction recall testing. Skin conductance responses (SCRs) served as the measure of learned fear. SCR magnitudes during conditioning, extinction, and extinction recall were all entered into correlations with resting regional brain metabolism previously quantified by FDG PET uptake in an unrestricted whole brain statistical parametric mapping (SPM8) analysis. In addition, region of interest analyses were performed on the fMRI data to examine whether prior resting metabolism in the dACC, vmPFC, or amygdala predicted functional activation of these regions during extinction recall.

Results: Fear conditioning led to significant differential SCR responses. The next day, the CS+E elicited significantly lower SCRs than the CS+U. Higher resting metabolism in the dACC predicted both higher conditioned responses during fear learning and poorer extinction retention. The ratio of vmPFC to dACC resting metabolic activity positively predicted functional activation in vmPFC during extinction recall. In contrast, resting metabolism in amygdala negatively predicted vmPFC activation during extinction recall.

Discussion: Resting regional brain metabolism may serve as a biomarker for fear learning and its inhibition via extinction.

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